THE UNIVERSITY OF MANITOBA

The Effect of Photoperiod and Temperature on the Induction, Maintenance, and Termination of Embryonic Diapause in Aedes vexans (Meigen) (Diptera:Culicidae)

by

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THE EFFECT OF PHOTOPERIOD AND TEMPERATURE ON THE INDUCTION, MAINTENANCE, AND TERMINATION OF EMBRYONIC DIAPAUSE IN AEDES VEXANS (MEIGEN) (DIPTERA:CULICIDAE)

by

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ABSTRACT

The Effect of Photoperiod and Temperature on the Induction, Maintenance, and Termination of Embryonic Diapause in Aedes vexans (Meigen) (Diptera:Culicidae).

Laboratory experiments conducted under controlled photoperiodic and temperature conditions show that embryonic diapause in Ae. vexans is induced by environmental factors acting on the parent (P_1) female. While both photoperiod and temperature are identified as environmental stimuli to which the P_1 females are sensitive, photoperiod is regarded as the predominant influence on the induction of embryonic diapause via the P_1 female. A photoperiod of 12L:12D induces P_1 females to produce a high incidence of diapause eggs at temperatures of 25°, 23°, and 20°C. At similar temperature conditions, embryonic diapause is averted if P_1 females are maintained at 16L:8D during the interval between emergence and oviposition.

A sensitivity to temperature is demonstrated by both the P_1 females and the embryonic eggs. Ae. vexans females, maintained at a temperature of 20°C, produce diapause eggs at a longer daylength than do those females which are maintained at 23°C. Further, the incidence of embryonic diapause is greater when both P_1 females and the deposited eggs are subjected to a moderately low temperature (20°C) than if either, or both, of the developmental stages are subjected to a moderately high temperature (23°C).

Diapause is sustained in eggs which are maintained at a short-photoperiod of 12L:12D and 20°C for a longer period of time than in eggs stored at a long-photoperiod of 16L:8D and 23°C. The influence

of photoperiod on embryonic diapause is masked at a high temperature ($30^{o}C$) or a low temperature ($5^{o}C$), i.e., photoperiodic influence on diapause is subordinate to the effect of extreme high or low temperature.

Exposure to a high temperature for a period of 7-14 days terminates the diapause condition in a high percentage of eggs. At $5^{o}C$, embryonic diapause is terminated *in toto* after 112 days; however, diapause is culminated in a significantly high percentage of eggs after 28 days at $5^{o}C$ provided that the eggs are conditioned at 16L:8D and $23^{o}C$ for 14 days following exposure to the low temperature.

The results of outdoor experiments provide evidence that P_1 females in Winnipeg, Manitoba ($49^{o}55'N$ lat.) produce diapause eggs during late July.

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CHAPTER I

INTRODUCTION

This thesis reports a study undertaken to determine the effect of temperature, photoperiod and maternal influence on the incidence of diapause in embryonated eggs of Ae. vexans. The results of these investigations are reported herein.

Aedes vexans is a multivoltine (2 or more generations per year) flood-water mosquito common to southern and central regions of Canada, as well as many areas of the United States, Europe and Asia. As is common in most multivoltine species, Ae. vexans exhibits a condition of facultative diapause. As autumn temperatures and daylengths decrease to a critical value, specific for the species at a given latitude, the eggs of Ae. vexans are induced to enter a period of diapause; a suppressed state of development and growth which ensures survival of the insect through adverse winter conditions. Diapause is terminated upon the return of favorable environmental stimuli in the spring; egg hatch (hatching of eggs) coincides with conditions suitable for growth and development of the immature insect.

Those eggs laid in the summer by Ae. vexans complete embryogenesis within a few days after deposition. The eggs will hatch at this time provided the oviposition sites are inundated and other hatching conditions are favorable. A temporary deviation of one or more environmental factors from the optimum produces the simplest type of dormancy in eggs, called quiescence. Reactivation from the quiescent state coincides with the return of favorable hatching conditions, which may be simply evaporation of the water in the pools and reflooding by rainfall.

Photoperiod is the most reliable environmental influence for programming seasonal changes and is utilized by insects as a diapause cue. Although it has little influence on the immediate well-being of the insect, photoperiod serves to synchronize the life cycle with the seasons (Lees 1955).

Ae. vexans has been incriminated as a vector of Western Equine Encephalitis in Alberta (Shemanchuk and Morgante 1968) and Manitoba (Sekla et al. 1980) and implicated as a possible vector of the virus in Saskatchewan by McLintock et al. (1970). The species is a known vector of dog heartworm, Dirofilaria immitis Leidy, 1856 (Jankowski and Bickley 1976; Hendrix et al. 1980), a nematode disease of dogs which may also infect man.

Ae. vexans has economic importance as a major pest of man and animals (McLintock 1944; Dixon and Brust 1972; Brust and Ellis 1976; Hudson and Gooding 1977) throughout much of the Canadian prairie-region and may be considered to be the most abundant and pestiferous mosquito found throughout its range within Canada. An overview of its economic status around the world has been provided by Horsfall et al. (1973).

Due to the ubiquitous nature of the species and the pest status it has attained, the majority of mosquito abatement programs within Canada have been designed to control Ae. vexans, or at least, to reduce populations of this species to a tolerable level either by larviciding or in extreme "outbreak" situations by adulticiding. Since it is a common practice for mosquito control personnel to assess the extent of potential Ae. vexans breeding sites during the fall of the year by systematic sampling of soil cores from those areas suspected of having concentrations

of mosquito eggs, it is essential that the circumstances surrounding the induction, maintenance, and termination of diapause in Ae. vexans eggs be understood. Without such an understanding the validity of such a sampling technique may be masked to a large degree.

There is a paucity of literature pertaining to the physiological and biochemical response mechanisms of insects as related to environmental stimuli and the over-wintering condition of diapause. This is particularily evident in studies of Aedes mosquitoes inhabiting the northern temperate regions where climatic conditions are varied and not uncommonly severe. This lack of information may be, in part, due to the absence of stenogamy (mating in a confined space) in most northern species, which has been a very significant factor in discouraging studies of these species (Brust 1971). Due to the difficulties in attaining conditions conducive to mating of these species in the laboratory, research material must be repeatedly collected in the field to replenish laboratory stocks or maintained via artificial insemination of adult female mosquitoes in the laboratory as first described by McDaniel and Horsfall (1957). The latter technique, albeit labour intensive, enables the researcher to utilize material of known age and life history for experimental purposes.

Most of the experiments performed for the current study were conducted in a manner which would demonstrate the importance of separate examination of eggs obtained from individual females during investigations into physiological responses of Aedes sp. mosquitoes. It was recognized, during experiments which precluded this study, that variables in embryonic physiological responses existed that could only be attributed to maternal influence, i.e., it was not uncommon, particularly at photoperiods of less than 16L:8D, for individual Ae. vexans females to produce compliments

of eggs that were neither diapause nor non-diapause *in toto*. The discovery of such a variability in embryonic response led to the realization that, to accurately assess the physiological responses of adult female populations to environmental stimuli, it was necessary to examine separately each egg batch produced by individual females of known age and life history. As well, one experiment was designed to delineate the phenological responses of *Ae. vexans* populations, subjected to natural photoperiodic and temperature conditions, in order to facilitate a comparison and correlation with physiological responses produced under laboratory conditions.

Other experiments conducted for this study were designed to demonstrate the physiological responses of diapause embryos to various photoperiodic and temperature regimes. Consideration is given to diapause 'development', intensity, duration and termination in an effort to explain the significant role of environmental stimuli in the overwintering of *Ae. vexans* eggs.

CHAPTER II

LITERATURE REVIEW

Diapause

The term "diapause" was originally coined by Wheeler (1893) to describe a stage during blastokinesis when growth of an insect embryo was arrested. Henneguy (1904) suggested use of the term to describe the arrest of growth for all stages during the life of an insect. Rouband in 1919 (cf. Wigglesworth 1965) separated the higher Diptera into two categories: "homodynamic" insects which are temporarily dormant due to the direct action of unfavorable environment, and "heterodynamic" insects which are subjected to a prolonged dormant period independent of the environment. Shelford (1929) later described growth retardation due to the effect of unfavorable environment as "quiescence". In cases where insect activity or development was arrested "spontaneously", Shelford applied the term dormancy or "diapause". Lees (1955) suggested that a distinction between "quiescence" and "diapause" could be made by considering the immediacy of response to environmental factors. However, since the immediacy of diapause induction and termination is dependent upon ecological factors and inherent species characteristics, there remains much confusion as to the distinction between the two phenomena. The physiological mechanisms which control dormancy may be quite diverse; quiescence and diapause are the two most important mechanisms involved, however only diapause appears to be controlled by a photoperiodic clock (Saunders 1976).

The generally accepted definition of diapause is that state of retarded or suppressed growth, resulting from the environmental

triggering of neuroendocrine mechanisms, which is induced well before adverse environmental conditions set in and which is maintained for some time irrespective of environment (Harvey 1962; Muller 1970; Mansingh 1971; Saunders 1976). Rarely is the diapause state accompanied by morphological characteristics that permit immediate easy identification of either diapausing or non-diapausing insects. Quiescence, the simplest type of dormancy, is produced by a sudden, unanticipated, non-cyclic, and usually short-duration deviation of one or more environmental factors from the optimum (Mansingh 1971). The termination of the quiescent state usually occurs shortly after the return of favorable environmental factors (Saunders 1976).

Diapause may be either obligatory or facultative. Univoltine* insect species exhibit an obligatory diapause that occurs automatically in each generation and which is presumably free of environmental control. Beck (1968) attributed this response to genetically controlled internal mechanisms. Facultative diapause, where growth retardation is either induced or averted depending upon the environmental conditions, is exhibited by multivoltine species. While Mansingh (1971) has stated that there is no significant physiological difference between obligatory and facultative diapause within the diapausing generation of multivoltine and univoltine species, other investigators (Muller 1970; Thiele 1973) support the view that a clear physiological differentiation does exist between the two types of diapause. Further, it is argued by Thiele (1973) that there is no simple connection between geographic distribution

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*Voltinism derives from Italian sericulture: volta-turn, time. Zool. The frequency or number of annual broods or generations.

and dormancy type as suggested by Mansingh (1971). Consequently, the dissension between ecologists and physiologists regarding the classification of insect diapause precludes elucidation of phylogenetic characteristics, based on diapause types, associated with geographical distribution of insect species.

Although both temperature and photoperiod are token environmental signals which correlate the growth and development of insects with the favorable seasons (Lees 1955), photoperiod is the only environmental factor which precisely relates the daily and seasonal rhythms of weather and climate (de Wilde 1962). Photoperiod thus serves to synchronize the insect life cycle with the seasons.

The action of photoperiod cannot be regarded as immediately favorable or unfavorable; the stimulus does not act directly upon the tissue cell, but rather exerts its influence through the nervous system and endocrine organs (Danilevskii 1965; Mansingh 1971). It is neither the absolute duration of photophase nor scotophase that appears to control diapause, but rather the duration of each relative to the other (Lees 1955; Corbet 1956; Danilevskii 1965; Adkisson 1966; Beck 1968; Saunders 1976). The decision to diapause or continue development is based on daylength measurements made by an endogenous circadian clock during the photosensitive stage of the insect's development; the induction of diapause is dependent on the occurrence of a fixed number of short-day photoperiod cycles during the photosensitive period (Saunders 1976). The concept of a required day number or RDN, proposed by Saunders (1976), has been confirmed for the mosquito, Ae. atropalpus, by Beach and Craig (1977) and Beach (1978).

At some point (critical photoperiod) on the photoperiodic response curve of a particular insect species, the incidence of diapause within the insect or insect population changes from high to low or vice versa (Beck 1968). The position of the critical photoperiod, specific to an insect population, is influenced by the temperature at which the development of light-sensitive stages takes place (de Wilde 1962; Danilevskii 1965). Thus, regardless of the constancy of seasonal cues provided by photoperiod, there may be much variation in the dates of the onset of diapause from year to year due to temperature conditions. Further, the influence of ambient temperatures, in conjunction with photoperiod, may serve to isolate geographical populations of an insect species on the basis of inherited or phenotypical adaptations to critical daylengths; an insect species may consist of many geographical populations, each exhibiting a characteristic critical photoperiod (Danilevskii 1965).

The duration of diapause provides some measure of the "intensity" of diapause in an insect species (Lees 1955) and is generally thought to be inversely proportional to the metabolic and developmental rates, or directly proportional to the degree of suppression of these rates (Beck 1968). The intensity of a photoperiodic-induced diapause response, as well as the effect of temperature on the response and the critical photoperiod, may differ according to the geographical latitude of local populations of an insect species (Way 1962); diapause intensity tends to be greater in insect populations inhabiting higher latitudes (Vinogradova 1960; de Wilde 1962; Danilevskii 1965; Depner and Harwood 1966).

Very little is known about the mechanisms by which either temperature or photoperiod hasten the completion of the diapause condition. The

physiological relationships, for the most part, have not been investigated fully. The concept of "diapause development" has been defined by Andrewartha (1952) as the physiological development that occurs during diapause in preparation for the resumption of morphogensis. Mansingh (1971) lends support to the view that diapause development or the "refractory phase" of diapause brings about the "activated phase" or the physiological ability to reactivate endocrine activity in diapausing individuals. The resumption of neuroendocrine activity appears to take place only under favorable environmental conditions with all physiological and biochemical developmental processes being gradually restored to a 'normal' state. However, diapause is not terminated immediately upon the return of favorable conditions; the rate of diapause termination is dependent upon the activated condition of the individual and the nature of the stimuli (Mansingh 1971).

High temperatures tend to avert or eliminate the diapause response (Lees 1955; de Wilde 1962; Danilevskii 1965; Beck 1968; Anderson 1968, 1970; Kalpage 1970; McHaffey and Harwood 1970; among others). In many instances, both high and low temperature extremes tend to mask the photoperiodic responses or shift the critical photoperiod to a point further along the response curve. Generally, when high temperatures coincide with scotophase (dark) the intensity of the diapause response is lessened and when low temperatures coincide with scotophase the intensity of the diapause response is increased (Danilevskii 1965; Beck 1968).

Few studies describe the effect of photoperiod and temperature with respect to maternal influence on embryonic diapause of mosquito species. The first report of the sensitivity demonstrated by the adults of the parental generation to photoperiodic stimulation was that of Vinogradova

(1965). Subsequent reports (McHaffey and Harwood 1970; Wilson and Horsfall 1970; McHaffey 1972a, 1972b; Pinger and Eldridge 1977) have confirmed that photoperiodic induction of embryonic diapause in various mosquito species is initiated, either in whole or in part, during the adult stage of the parental generation.

Diapause in Aedes vexans

There are several accounts in the literature that report the influence of photoperiod and temperature on diapause in Ae. vexans. Historically, it was held that temperature was the major environmental influence on the induction of diapause in Ae. vexans eggs. However, the results of recent investigations provide evidence that photoperiod is, in large part, the major influence on the induction of embryonic diapause and that embryonic diapause is a consequence of the photoperiod to which the parent female was subjected. For the purpose of this review, the aforementioned studies will be described as either embryonic induction or maternal influence.

i) Embryonic Induction Of Diapause

It has been reported by a number of investigators (Dyar 1902; Gjullin et al. 1950; Horsfall et al. 1973) that Ae. vexans eggs, collected from field sites during autumn, demonstrated an erratic hatching response when subjected to a hatching stimulus. Horsfall et al. (1973) have attributed such an erratic response to the fact that many of the eggs have entered a latent state due to the effect of cold weather; to remove latency, the eggs must be preconditioned at a temperature of 25^{o}C for at least 15 days prior to immersion in the hatching medium.

Laboratory studies by Horsfall et al. (1973) have shown that young eggs (<25 days old) could be induced to enter a latent state if exposed to any temperature below 25°C for a period of 7 days or more. As was the case for field-collected eggs, many of the latent eggs could be 'conditioned' to hatch if they were subjected to 25°C for a period of 14 days prior to immersion in a hatching medium. It is of interest to note that latent eggs which had been stored at 10°C for 21 days responded more rapidly to a hatching stimulus, after return to 25°C, than did those eggs which were stored at 18°C for a similar period of time and also returned to 25°C. The rapid recovery from a latent state exhibited by those eggs which had been stored at a low temperature (10°C) would enable this species to develop in northern climates. Similarly, failure to recover from latency exhibited by many of those eggs stored at a more moderate temperature (18°C) would ensure survival of at least a portion of the population in areas of variable climatic conditions where extreme temperature fluctuations might prevent or hinder the development of larval, pupal, or adult populations should there be a significant decrease in temperature following egg hatch. Horsfall et al. (1973) have reasoned that the ability of embryos to remain latent at intermediate ranges of temperature, as well as low temperatures, would allow this species to inhabit the wide ranges in latitude known for the distribution of the species.

While it has been demonstrated that new eggs can be induced to enter a latent state by exposure to cold, Horsfall et al. (1973) have shown that older eggs (≥230 days old) cannot be influenced by exposure to reduced temperatures (4°, 8°, 10°, and 23°C) for periods of up to four

weeks. Since it is not uncommon for Ae. vexans eggs to remain viable for a period of two or more years under field conditions (Horsfall et al. 1973; among others), the durability of eggs is of considerable ecological significance when it is considered that such eggs are able to withstand low temperatures for an extended period of time without the benefit of a latent physiological state.

Field studies by Breeland et al. (1965) have indicated that a latitudinal variation in hatching exists between populations of Ae. vexans eggs from Alabama and Minnesota. Eggs from each state were collected in September and placed together in suitable sites at each location. Periodic samples from each population were retrieved throughout the fall and winter and exposed to a hatching stimulus. In all experiments, whether with eggs derived from sod samples or with eggs obtained from isolated females, the Minnesota eggs hatched less readily in the fall and early winter (September 14 - January 4) than did Alabama eggs. Thereafter, hatching response in eggs from both populations was similar. Breeland et al. (1965) have suggested that a conditioning period may be required to alleviate the hatching resistance of Minnesota eggs after the inception of cooler weather.

During a 5-year study, Cook and Buzicky (1971) determined that a reduction in egg hatchability for Ae. vexans began as early as mid-August in St. Paul, Minnesota. A gradual decline in hatchability continued until the middle of October, at which time no eggs could be induced to hatch. However, it was not determined if temperature, photoperiod, or a combination of both accounted for the decline in egg hatch.

Following a relatively intensive laboratory study, McHaffey (1972b) concluded that temperature was the primary factor in determining whether diapause was induced or averted in eggs from 16L:8D female Ae. vexans collected in central Washington (46°N.lat.). Further, it was determined that a short-photoperiod (11L:13D) produced significantly more diapause eggs than did a long-photoperiod (16L:8D) at 10°C. However, at temperatures of 25° and 32°C very few diapause eggs were produced at either a long- or short-photoperiod regime. A decreasing temperature regime (32°-25°-10°C) was found to be most suitable for producing diapause eggs, particularily if the eggs were stored at a short- rather than a long-photoperiod. McHaffey (1972b) suggested that a maximum number of eggs are induced to enter diapause as a result of the seasonal cues provided by decreasing temperatures and a short-photoperiod.

Khelevin (1961), working in the USSR, has attributed the induction of diapause in Ae. vexans eggs to decreasing temperatures only. No mention of photoperiodic cues was made in his report.

ii) Maternal Influence On Diapause

Laboratory experiments conducted by Wilson and Horsfall (1970) have demonstrated the effects of maternal influence on the hatchability of Ae. vexans eggs. It was found that the hatchability of the eggs was decreased when the parent females were exposed to a short-photoperiod (12L:12D), whereas eggs oviposited by females which were exposed to a long-photoperiod (16L:8D) hatched in toto. Although a critical photoperiod was not determined during their investigation, an observation made by Horsfall et al. (1973) suggested that the natural photoperiod occurring during the first part of August, in Illinois, would induce

some females to deposit diapause eggs. A low order of hatchability in Ae. vexans eggs oviposited in the laboratory, under natural light, during the interval of August 15-20 was noted by these workers. This finding further substantiates the theory of maternal influence in Ae. vexans put forward by Wilson and Horsfall (1970).

McHaffey (1972b) stated that the hatchability between groups of Ae. vexans eggs, collected from females captured during 16L:8D, 14½L:9½D, and 13½L:10½D natural photoperiods, varied considerably. Despite variable results, McHaffey suggested that a maternal influence on egg hatchability does exist since the eggs from 14½-hour females were more responsive to long-(16L:8D) and short-(11L:13D) photoperiods than were eggs from 16- and 13½-hour females. Interestingly, eggs from 13½-hour females exhibited a relatively high order of hatchability, with little variation, during this study. Unfortunately, all eggs collected from adults were randomized for these experiments, thereby preventing any observations on the response of eggs from individual females. Furthermore, McHaffey initiated his experiments with eggs that were less than 7 days old. The variability in hatching response may well have been attributable to factors other than photoperiod.

CHAPTER III

METHODS & MATERIALS

1. GENERAL METHODS

Collection and Storage of Study Material

The original material used for this study was collected from sites in or near Winnipeg, Manitoba during June, July, and August, 1978. On each occasion, several hundred blood-seeking Ae. vexans females were collected while attempting to bite the author. These were returned to the laboratory, fed blood and isolated for oviposition in single-female oviposition cages as described by Kalpage and Brust (1974). Details of egg storage and the colonization of Ae. vexans are outlined in Chapter III, part 2. During 1979, adult collections were made at the above sites for the purpose of replenishing the laboratory colony.

All laboratory experiments were conducted in BOD incubators or temperature-controlled water baths. The BOD incubators were equipped with an incandescent light source (15W bulb) and a time clock for photoperiod control. A constant temperature was maintained, during the dark and lighted part of the photoperiod cycle at $\pm$ 1°C of the set temperature. Relative humidity was maintained at 75 $\pm$ 5%.

The water baths were similar to the one described by Brust (1967). Partitions were placed inside the baths, providing light-tight compartments which could be used for photoperiod studies. Each compartment was equipped with a light source (12-volt, miniature bulb*) and a time

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*Spectro miniature lamps, no. 1855. Spectro Electric Industry Inc., 1774 Midland Ave., Scarborough, Ontario M1P 3C2.

clock for photoperiod control. A constant temperature, within $\pm$ 0.5°C of the set bath temperature, was maintained within each compartment.

Field experiments were conducted in a small insectary (48cm x 22cm x 42cm high) located next to the laboratory building (Fig. 1). The insectary was constructed of 2 x 4 cm lumber, to which mosquito screening was attached, and positioned to avoid direct sunlight. It was found that the screening prevented access to the study material by birds and other unwanted animals. The sloping transparent-fiberglass roof (3mm thick) allowed light penetration, yet was sturdy enough to provide protection from the elements for the instruments inside the insectary. Two doors on the front of the insectary provided easy access to the study material.

Continuous temperature records were kept in the insectary with a Weksler^{R} temperature recorder. Relative humidity was monitored with a Bendix^{R} hygrothermograph (model #594). A continuous recording photometer, similar in principal to the one described by Callahan (1964), provided a record of daily photoperiod regimes for comparison with Smithsonian Meteorological Tables (Vol. 114 1966).

2. COLONIZATION TECHNIQUES

The following sections describe handling and rearing procedures used for the various developmental stages of Ae. vexans.

(a) Eggs

For all experiments, eggs were collected from individual females which had been isolated for oviposition in single-female oviposition cages as described by Kalpage and Brust (1974). The oviposition substrate consisted of circular pads of paper towelling which were placed

Figure 1. Outdoor insectary containing a Weksler[R] temperature recorder, a Bendix[R] hygrothermograph, plastic pans containing single-female oviposition cages, and a large petri dish used for storage of Ae. vexans eggs. The continuous recording photometer is not shown in this photograph.

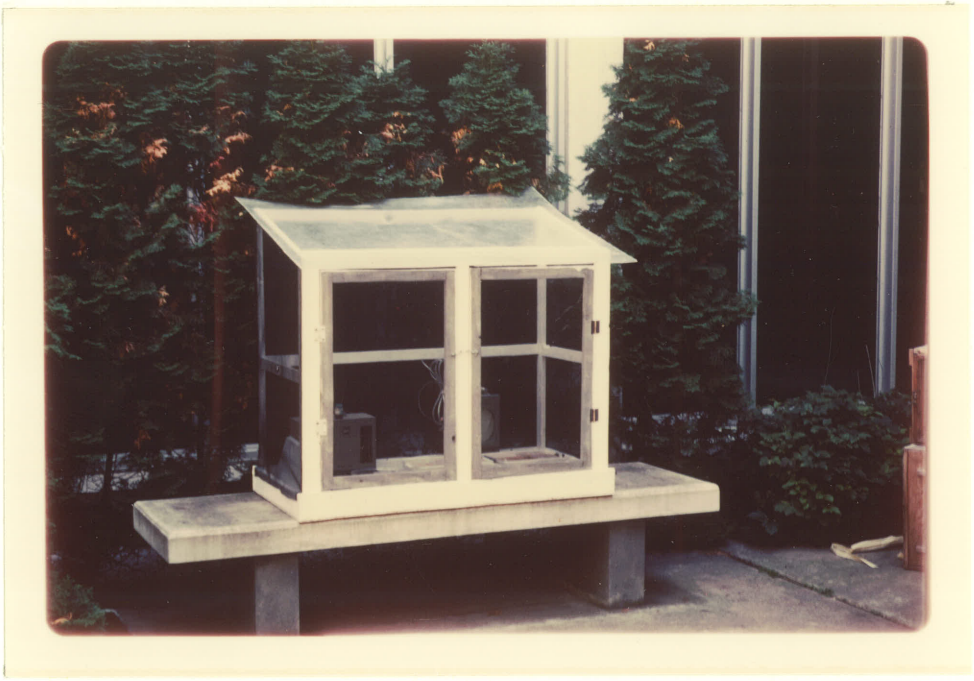

Fig. 1

under each cage on a larger pad of moist paper towelling.

Eggs were collected daily, washed, placed on individual nylon pads and stored on moist glass wool in covered petri dishes. This technique kept eggs moist and drastically reduced contamination by molds and fungi (Kalpage 1970). The petri dishes were labelled and stored in BOD incubators at appropriate photoperiodic and temperature regimes.

Eggs on pads were hatched in a nutrient broth solution (2:1000 w/w, powder in tap water) at room temperature. All eggs that failed to hatch after 16 hours in the nutrient broth solution were bleached (cf. Tripis 1970) and examined to determine viability. Those eggs containing fully formed whitish embryos with eye spots, a hatching spine and abdominal segmentation were considered to be viable but in a state of diapause. Non-embryonated eggs and eggs with brownish embryos were discarded.

In order to check egg samples for viability prior to the initiation of an experiment, a few (5-10) eggs from each pad were dissected and examined for embryonation. Those pads of eggs with a low percentage viability (<50%) were discarded.

(b) Larvae

Larvae were transferred from vials of nutrient broth solution to covered plastic pans (22cm x 30cm x 6cm deep) containing ca. 1000ml of dechlorinated tap water. To minimize the detrimental effects of crowding, only 100 larvae were placed in each pan.

Larval diet consisted of a few drops of a slurry of finely ground liver powder* and dechlorinated tap water. The daily amount of food

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*ICN Pharmaceuticals Inc., Cleveland, Ohio.

provided was dependent upon the instar stage of the larvae and water temperature. Excessive amounts of food caused fouling of the larval medium and greatly increased the incidence of larval mortality. Although larval development varied according to water temperature, there was no appreciable difference in survival rates at the rearing temperatures used (20^{o}, 23^{o}, and 25^{o}C).

(c) Pupae

Pupae were removed from the larval pans daily and were transferred to 300ml styrofoam food containers (100 pupae per container) half-filled with dechlorinated tap water. The pupal containers were placed in acrylic cube cages (18cm per side) in which the adults emerged.

Paper wicks, moistened with sugar water, provided the necessary food and water requirements of the adults. The wicks helped to maintain a relative humidity of 70-80% inside the cages.

(d) Adults

Emergent adults were held in the acrylic cages for an interval sufficient to allow maturity of both sexes. Although efforts to induce Ae. vexans to mate in cages under laboratory conditions have met with partial success (Taylor and Brust 1974), the requirements of this present study were such that a sufficient population of young mated females could not be provided by this technique. A continuous colony of Ae. vexans was maintained through induced insemination of female imagos. The technique used was a modification of the method reported by Horsfall and Taylor (1967).

Healthy males, five to seven days old, were found to be the most vigorous and suitable for mating, while three to four day old females proved to be very receptive to insemination. The rearing technique,

described for larvae and pupae, provided a relatively synchronous emergence of males two to three days prior to a similarily synchronous emergence of females. It was found that such a rearing method facilitated the use of adults of both sexes that were of uniform age.

Rates of insemination for Ae. vexans females, used in these experiments, were consistently high (85-95%).

3. INDUCTION, MAINTENANCE, AND TERMINATION OF DIAPAUSE

Investigations on the induction, maintenance, and termination of diapause were carried out using temperature and photoperiod as the primary experimental variables.

(a) Induction of Diapause

Preliminary experiments revealed that there was a high incidence of diapause in those eggs deposited by females which had been maintained at a short-photoperiod (12L:12D). Conversely, those eggs deposited by females which had been held at a long-photoperiod (16L:8D) exhibited little or no diapause.

Induction Experiment 1. The first experiment was designed to determine the effect of photoperiod on different stadia of Ae. vexans at 20° and 23°C. A short-photoperiod (12L:12D) and a long-photoperiod (16L:8D) were used. Specific stages of the developmental cycle (larvae, pupae, adults, and/or eggs) were transferred from the short-photoperiod to the long-photoperiod, and vice versa. After 21 days at the predetermined photoperiod, the eggs, deposited by adult females used in these experiments, were immersed in nutrient broth solution for 16 hours. The number of larvae, unhatched viable eggs and non-viable eggs were recorded

for each treatment.

In conjunction with this experiment, a study was initiated to determine the incidence of installment hatching in those eggs which were maintained at 23°C. Ten egg batches (each egg batch represents the eggs from one female) from each combination of photoperiod treatment were subjected to three separate hatch attempts. The eggs were maintained at each photoperiod treatment for 21 days prior to the initial flooding in nutrient broth; all unhatched eggs were returned to storage at 16L:8D and 23°C for an additional 7-day period. Subsequently, the eggs were flooded for a second time and the number of larvae recorded. As before, any unhatched eggs were returned to the 16L:8D incubator (23°C) for an additional 7 days of storage before flooding a third time. The number of eggs hatched after each flooding, as well as the total number of eggs hatched after three flooding attempts, were recorded.

Induction Experiment 2. A second experiment was conducted to determine the interval required, after oviposition, for the onset of embryonic diapause. Eggs, obtained from 12L:12D females, were stored at 16L:8D for a period of up to 21 days. At predetermined intervals of 8, 10, 12, 14, 16, 18, and 21 days after oviposition, five to seven egg batches were removed from the storage dishes and flooded in nutrient broth. The following day, the number of larvae and unhatched viable eggs in each sample were recorded. This study was carried out using egg storage temperatures of 20° and 23°C.

Induction Experiment 3. For the third experiment, an investigation was undertaken to determine the daily photoperiod(s) capable of inducing embryonic diapause at three different temperatures. At each temperature

of 20°, 23°, and 25°C, populations of Ae. vexans were reared through to the adult stage under the influence of various photoperiods (12L:12D, 13L:11D, 14L:10D, 15L:9D, and 16L:8D). Eggs, deposited at each photoperiod, were transferred to 16L:8D incubators for storage at 23°C. At the end of a 21-day storage period, the eggs were subjected to a hatching stimulus.

In order to determine the effect of egg storage temperatures on the incidence of embryonic diapause, the above experimental design was altered slightly for two temperatures (20° and 23°C). Some of those eggs which had been deposited by females at 23°C, under the influence of each photoperiod regime, were transferred to a 16L:8D incubator which was held at 20°C. Similarily, some of the eggs which had been laid by females at 20°C, at each photoperiod, were transferred to a 16L:8D incubator at 23°C. Following a 21-day storage period, all eggs were subjected to a hatching stimulus.

Induction Experiment 4. The last experiment in this series was conducted outdoors in order to determine the influence of natural photoperiodic and temperature regimes on the incidence of embryonic diapause. Populations of Ae. vexans were reared through to the adult stage under natural photoperiodic regimes in the laboratory (23°C) to facilitate the use of adults of uniform age for mating and blood-feeding. The females of each population were mated; subsequently, each population was divided equally into groups A, B, and C. Group A females were provided with a blood meal seven days after emergence, isolated for oviposition, and transferred from the laboratory to the outdoor insectary, along with the other non-blood-fed groups. Group B females were provided with a

blood meal seven days later, isolated for oviposition, and returned to the insectary. In like fashion, Group C females were blood-fed seven days after Group B females, isolated for oviposition and returned to the insectary.

Four populations, nos. I, II, III, and IV, of Ae. vexans females were handled in the above manner. All eggs deposited by these females were collected daily and stored on nylon pads in covered petri dishes, in the insectary, for 21 days prior to immersion in nutrient broth. Egg samples from two groups of females (IV *B and IV *C) were collected and stored for 21 days in a laboratory incubator set at 16L:8D and 23°C in order to assess the effect of a storage temperature, higher than the outdoor temperature, on the incidence of embryonic diapause.

Two populations, nos. V and VI, were reared through to the adult stage and maintained at 16L:8D for seven days after emergence in the laboratory. Group A females of each population were blood-fed on the seventh day and then transferred, along with the non-blood-fed Group B females, to the insectary. Seven days later, Group B females were provided with a blood meal and returned to the insectary for oviposition. No Group C was established in this study. All eggs were stored in the insectary for a period of 21 days prior to immersion in nutrient broth.

One group of Ae. vexans adults, designated as Population VII, was reared through to the adult stage and maintained in the laboratory (23°C) under a natural photoperiodic regime. Mated females were provided with a blood meal seven days following emergence and allowed to oviposit. Eggs were collected daily and stored, under similar conditions, for 21 days prior to immersion in nutrient broth.

(b) Maintenance of Diapause

Having determined the critical photoperiod and the developmental stage responsive to photoperiod for Ae. vexans, the following experiments were performed to determine the intensity of diapause in eggs stored for various periods of time.

Maintenance Experiment 1. For this experiment, batches of 21-day old diapause eggs, obtained from 12L:12D females at 23°C, were flooded in nutrient broth to eliminate any non-diapause eggs from the experimental material. Unhatched eggs were then stored in either 16L:8D or 12L:12D incubators set at 20°C for predetermined intervals of 28, 42, 56, 70, 84, 98, and 112 days before immersion in nutrient broth solution.

Maintenance Experiment 2. Four-week old diapause eggs, obtained from field-collected females and maintained at 20°C, were used in this experiment. Eggs from individual oviposition pads were pooled together, randomly selected for transfer to nylon pads in covered petri dishes, and placed in 20°C incubators set at either 16L:8D or 12L:12D for storage. At predetermined intervals of 7, 14, 28, 56, and 112 days, 6 samples (of 30 eggs each) from each photoperiod, were immersed in nutrient broth for 16 hours. Unhatched eggs were then returned to their original pads in covered petri dishes and stored at 16L:8D and 23°C for an additional 14-day post-treatment period. At the end of the second treatment period, the eggs were reflooded in nutrient broth solution. The number of larvae and unhatched viable eggs were recorded for each flooding after each treatment period.

(c) Termination of Diapause

The following experiments were carried out with diapause eggs in order to assess the effect of low and high temperatures on the photoperiodic responses of diapausing embryos.

Termination Experiment 1. Four-week old diapause eggs, obtained from field-collected females and maintained at 20°C, were pooled and randomly selected for transfer to nylon pads (30 eggs per pad) in covered petri dishes. The egg samples were then stored in 5°C incubators set at either 16L:8D or 12L:12D for treatment periods of 7, 14, 28, 56, and 112 days. At the end of each treatment period, the eggs were transferred to a 16L:8D incubator (23°C) for 48 hours prior to immersion in nutrient broth solution. Unhatched eggs were returned to the 16L:8D and 23°C incubator for an additional 14-day post-treatment period. At the end of this treatment period, the eggs were reflooded in the hatching medium.

The number of larvae and unhatched viable eggs were recorded for each flooding after each treatment period.

Termination Experiment 2. Batches of 28-day old diapause eggs, obtained from 12L:12D females at 23°C, were flooded in nutrient broth to eliminate any non-diapause eggs from the samples to be used in this experiment. Unhatched eggs were returned to their original pads in covered petri dishes, transferred to a water bath maintained at 30°C, and subjected to either a 16L:8D or 12L:12D photoperiod. At predetermined intervals of 7, 14, 21, and 28 days, egg samples (six or seven batches) were removed from the water bath and placed at room temperature (23°C) for 1 hour before immersion in the hatching medium.

The number of larvae and unhatched viable eggs were counted the next day.

Termination Experiment 3. As described for 'Termination Experiment 1', four-week old diapause eggs were used for this experiment. However, egg samples were stored in a 30°C water bath, rather than 5°C incubators, at photoperiods of 16L:8D or 12L:12D for treatment periods of 7, 14, 28, 56, and 112 days. At the end of each treatment period, the eggs were transferred to a 16L:8D incubator (23°C) for 48 hours prior to immersion in nutrient broth solution. Any unhatched eggs were returned to the 16L:8D and 23°C incubator for an additional 14-day post-treatment period. At the end of this storage period, the eggs were reflooded in the hatching medium.

The number of larvae and unhatched viable eggs were recorded for each flooding after each treatment period.

CHAPTER IV

RESULTS

1. INDUCTION OF DIAPAUSE

(a) Effect of short-day (12L:12D) and long-day (16L:8D) photoperiods, applied to the developmental stages of Ae. vexans at 20°C, on the induction of embryonic diapause.

It is apparent from the results of these experiments (Table I) that those females subjected to a short-daylength, at either temperature, produced a significantly higher percentage of diapause eggs than did those females subjected to a long-daylength. Although the P_1 females exhibit a strong response to photoperiod, it is evident that the pupal stage is also responsive to photoperiod; particularly, as is noted in the short-daylength treatment at 23°C. A high variability in egg hatch response is shown when the pupal stage is subjected to a short-daylength at 20°C.

With the exception of the treatment in which only the larval and pupal stages were subjected to a short-daylength (treatment 5, Table I), the percentage egg hatch was consistently higher at 23°C than at 20°C for all combinations of photoperiodic treatments used.

(b) Hatching response of Ae. vexans eggs to repeated floodings at 23°C

This experiment was carried out, in conjunction with the preceding experiment at 23°C, in order to determine if the experimental design for the assessment of the diapause condition in Ae. vexans eggs should include repeated floodings.

As the results in Table II indicate, virtually no egg hatch was found to occur as a result of a second or third hatch attempt. All

TABLE I

Effect of short-day (12L:12D) and long-day (16L:8D) photoperiods, applied to the developmental stages of Ae.vexans at 20°C and 23°C, on the induction of embryonic diapause. All eggs stored at designated photoperiod for 21 days prior to immersion in hatching medium.

Developmental stages exposed to photoperiod		23°C			20°C		
12L:12D	16L:8D	No. Egg Batches[1]	No. Viable Eggs	% Hatch Mean ± S.E.	No. Egg Batches	No. Viable Eggs	% Hatch Mean ± S.E.
E	1,2,3,4,P,A[2]	19	1419	98.4 ± 0.77 $_{c}$[3]	20	931	[4] $_{s}$ 72.7 ± 4.13 $_{b}$
A,E	1,2,3,4,P	20	1213	23.3 ± 5.91 $_{a}$	21	829	$_{s}$ 0.0 ± 0.00 $_{a}$
P,A,E	1,2,3,4	30	1723	27.4 ± 3.38 $_{a}$	14	753	$_{s}$ 4.3 ± 3.90 $_{a}$
1,2,3,4	P,A,E	27	1125	97.8 ± 0.69 $_{c}$	16	681	$_{s}$ 80.9 ± 5.70 $_{b}$
1,2,3,4,P	A,E	20	1199	73.5 ± 6.54 $_{b}$	11	610	72.0 ± 6.66 $_{b}$
1,2,3,4,P,A	E	23	1214	30.2 ± 4.98 $_{a}$	28	1261	$_{s}$ 7.4 ± 1.69 $_{a}$

1 Each egg batch represents the eggs oviposited by one female.

2 Numerals indicate larval instars; P= pupae, A= adults, E= eggs.

3 Common letter following column indicates no significant difference at 5% level between treatments. Student-Newman-Keuls test.

Table I (continued)

4 Letter *s* preceding column indicates a significant difference at 5% level between percentage egg hatch at different temperature treatments. Student-Newman-Keuls test.

Abbrevation: S.E.= standard error.

TABLE II

Hatching response of Ae.vexans eggs to repeated floodings at 23°C. All eggs stored at designated photoperiod for 21 days prior to first immersion in hatching medium.

Developmental stages exposed to photoperiod		No. Egg Batches[1]	No. Viable Eggs	1st Hatch Attempt (21 days)	2nd Hatch Attempt (+7 days)	3rd Hatch Attempt (+7 days)	Total % Hatch
12L:12D	16L:8D			No.Eggs Hatched	No.Eggs Hatched	No.Eggs Hatched	Mean ± S.E.
E	1,2,3,4,P,A[2]	10	1036	1031	0	0	99.5 ± 0.02 c[3]
A,E	1,2,3,4,P	10	691	192	0	1	30.9 ±10.40a
P,A,E	1,2,3,4	10	649	198	0	0	32.6 ± 8.19a
1,2,3,4	P,A,E	10	482	478	0	0	99.3 ± 0.07 c
1,2,3,4,P	A,E	10	674	353	0	0	62.5 ± 9.46 b
1,2,3,4,P,A	E	10	530	158	0	0	34.9 ± 7.42a

1 Each egg batch represents the eggs oviposited by one female.

2 Numerals indicate larval instars; P= pupae, A= adults, E= eggs.

3 Common letter following column indicates no significant difference at 5% level between treatments. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

non-diapause eggs hatched during the initial flooding. The total percentage egg hatch for each combination of photoperiodic treatment was found to correspond with the results shown for the preceding experiment (Table I).

(c) Effect of temperature on the onset of diapause in eggs from 12L:12D Ae. vexans females.

It was found during preliminary investigations that the onset of diapause in Ae. vexans eggs, obtained from short-photoperiod females, was related to the post-oviposition storage treatment. In order to assess this relationship, new eggs (<24 hours old) from 12L:12D females were stored at a temperature of either 20°C or 23°C for a period of up to 21 days. All eggs were stored at 16L:8D in order to allow a comparison of percentage egg hatch after 21 days with that found in previous experiments (Tables I and II).

As the results in Table III indicate, the onset of embryonic diapause is directly related to the post-oviposition storage period and is temperature dependent. Eggs flooded after a storage period of 8 or 10 days at 23°C were found to exhibit a significantly higher percentage hatch than did those eggs flooded after 16, 18, or 21 days. At 20°C, 10-day old eggs hatched more readily than did eggs stored for a longer period of time but less readily than eggs stored for only an 8-day period.

For all treatment periods, with the exception of the 8-day storage period, there was a significantly higher egg hatch at 23°C than at 20°C.

(d) Incidence of diapause in eggs from Ae. vexans females of three different temperature and five different photoperiod treatments.

The purpose of this experiment was to determine what range of photo-

TABLE III

Effect of temperature on the onset of diapause in eggs from 12L:12D *Ae. vexans* females. Eggs were held at 20°C or 23°C for designated storage period prior to immersion in hatching medium.

Egg Storage (Days)	23°C No. Viable Eggs	23°C % Hatch Mean ± S.E.	20°C No. Viable Eggs	20°C % Hatch Mean ± S.E.
8	562	84.4 ± 5.39*a*[1]	231	70.7 ± 5.68*a*[1]
10	335	[2]*s*81.9 ± 8.24*a*	128	42.2 ± 8.50 *b*
12	320	*s*59.2 ±12.63*ab*	283	12.2 ± 5.47 *c*
14	323	*s*53.9 ± 9.27*ab*	288	15.3 ± 7.90 *c*
16	378	*s*29.2 ± 8.06 *b*	224	4.5 ± 2.80 *c*
18	243	*s*31.6 ± 7.89 *b*	304	4.3 ± 2.59 *c*
21	186	*s*33.1 ± 9.39 *b*	283	6.2 ± 2.61 *c*

1 Common letter following column indicates no significant difference at 5% level between percentage egg hatch for different number of days of treatment. Student-Newman-Keuls test.

2 Letter *s* preceding column indicates a significant difference at 5% level between percentage egg hatch at different temperatures for the same number of days of treatment. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

periods and temperatures induced females to produce diapause eggs.

From the results summarized in Table IV, and depicted in Figs. 2, 3, and 4, it can be seen that diapause is averted in eggs deposited by females which were subjected to a photoperiod greater than 15L:9D at all temperatures. Conversely, diapause was found in a high percentage (<50%) of the eggs deposited by females which had been subjected to a photoperiod less than 14L:10D at any of the treatment temperatures. At a photoperiod of 14L:10D, significantly fewer diapause eggs were produced by females maintained at 25°C than by those females maintained at lower temperatures of 20° or 23°C.

(e) Effect of egg storage temperature on the incidence of diapause in the eggs of Ae. vexans females reared at different combinations of photoperiod and temperature.

From the results shown in Table V, and illustrated between Figs. 3 and 6, and Figs. 4 and 5, it was concluded that egg storage temperature influenced the hatchability of eggs produced by females in all photoperiod treatments. Most noticeable was the fact that there was generally a significantly higher incidence of diapause in those eggs stored at 20°C than in those eggs stored at 23°C.

Critical photoperiods for each temperature combination used in this experiment are shown in Figs. 3, 4, 5, and 6. As well, a critical photoperiod is plotted in Fig. 2 for that population of Ae. vexans of which the females were held at 25°C for oviposition and the subsequent eggs produced were stored at 23°C (as shown in Table IV). There is a considerable shift in the critical photoperiod determined for the various populations as a result of the temperature effect on both the eggs and the P_1 females. For that population of which the P_1 females were held

TABLE IV

Incidence of diapause in eggs from Ae. vexans females of three different temperature and five different photoperiod treatments. All eggs were stored at 16L:8D and 23°C for 21 days prior to immersion in hatching medium.

Rearing Photoperiod of females (L:D)	Rearing temperature for females (°C) 25			23			20		
	No. Egg Batches[1]	No. Viables Eggs	% Diapause Mean ± S.E.	No. Egg Batches	No. Viable Eggs	% Diapause Mean ± S.E.	No. Egg Batches	No. Viable Eggs	% Diapause Mean ± S.E.
12:12	28	1222	58.5 ± 5.60*a*[2]	29	1492	68.1 ± 4.27*a*	18	1100	52.3 ± 6.66 *b*
13:11	19	845	55.3 ± 7.97*a*	24	913	62.7 ± 6.17*a*	21	1089	73.3 ± 5.58*a*
14:10	35	1953	[3]*s*27.2 ± 5.20 *b*	31	1340	47.1 ± 5.59 *b*	33	1903	48.3 ± 5.71 *b*
15:9	20	1152	3.7 ± 1.44 *c*	24	1107	7.5 ± 3.23 *c*	45	2452	7.4 ± 1.95 *c*
16:8	38	2086	2.8 ± 0.86 *c*	27	1060	2.1 ± 0.75 *c*	45	2439	1.0 ± 0.27 *c*

1 Each egg batch represents the eggs oviposited by one female.

2 Common letter following column indicates no significant difference at 5% level between hatch response of eggs from females of different photoperiod treatments at each temperature. Student-Newman-Keuls test.

TABLE IV (continued)

3 Letter *s* preceding column indicates a significant difference at 5% level between hatch response of eggs from females of different temperature treatments at each photoperiod. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

Figure 2. The effect of photoperiod and temperature on the incidence of embryonic diapause of Ae. vexans. P_1 females were maintained at 25°C for each of the photoperiod treatments; all eggs were maintained at 23°C and 16L:8D for 21 days prior to flooding. The critical photoperiod required for induction of diapause is indicated by arrow on x-axis.

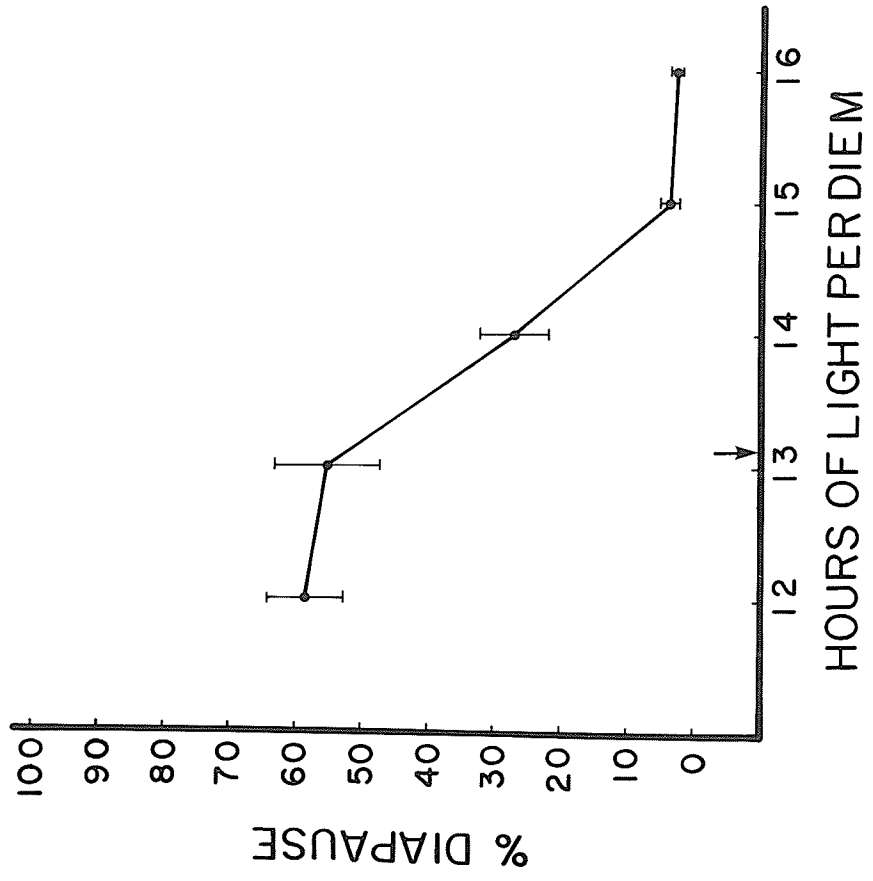
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Fig. 2

Fig. 3, 4. The effect of photoperiod and temperature on the incidence of embryonic diapause of Ae. vexans.
Fig. 3. P_1 females were maintained at 20°C for each of the photoperiod treatments; eggs were maintained at 23°C for 21 days prior to flooding.
Fig. 4. P_1 females were maintained at 23°C for each of the photoperiod treatments; eggs were maintained at 23°C for 21 days prior to flooding.
The critical photoperiod required for induction of diapause is indicated by arrow on x-axis of each graph.

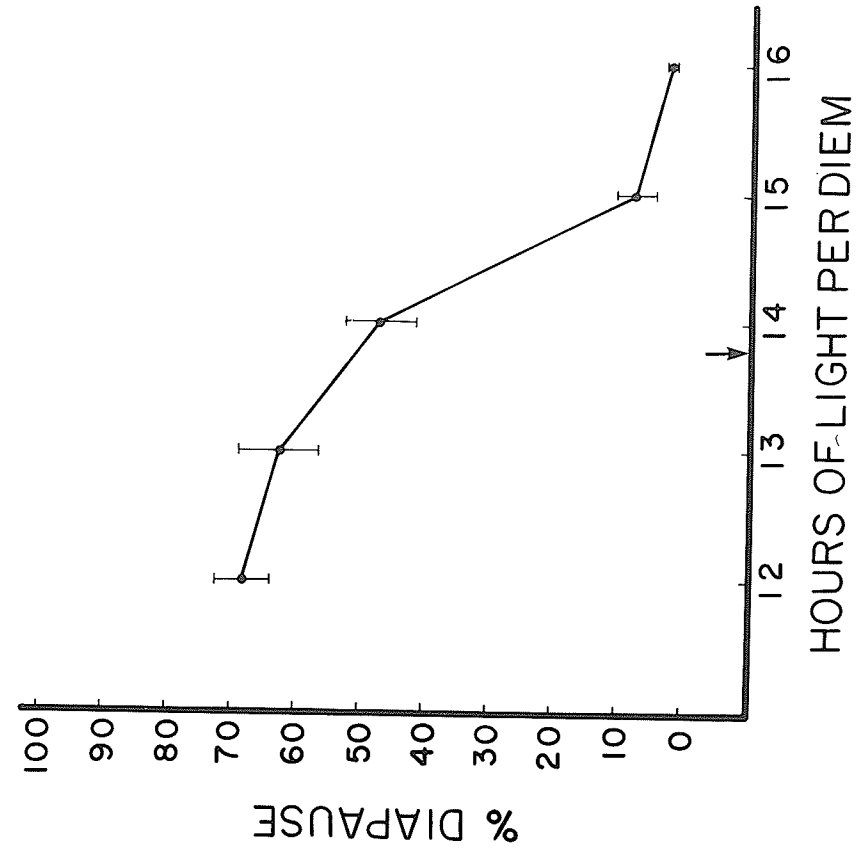
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Fig. 4

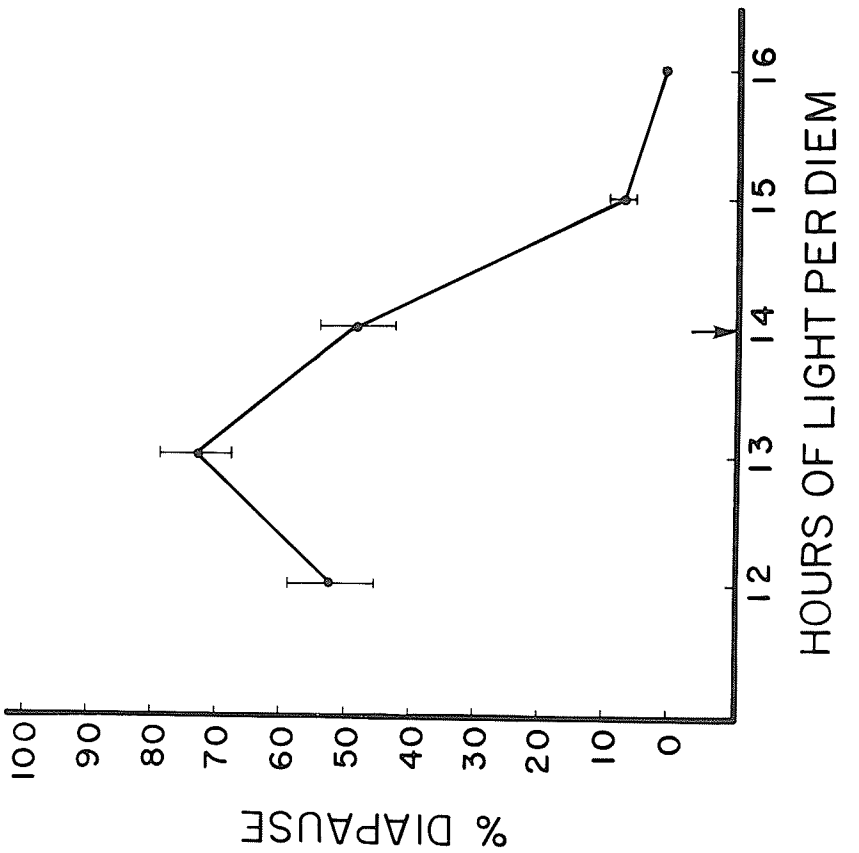
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Fig. 3

Fig. 5, 6. The effect of photoperiod and temperature on the incidence of embryonic diapause of Ae. vexans.
Fig. 5. P_1 females were maintained at 23°C for each of the photoperiod treatments; eggs were maintained at 20°C for 21 days prior to flooding.
Fig. 6. P_1 females were maintained at 20°C for each of the photoperiod treatments; eggs were maintained at 20°C for 21 days prior to flooding. The critical photoperiod required for induction of diapause is indicated by arrow on x-axis of each graph.

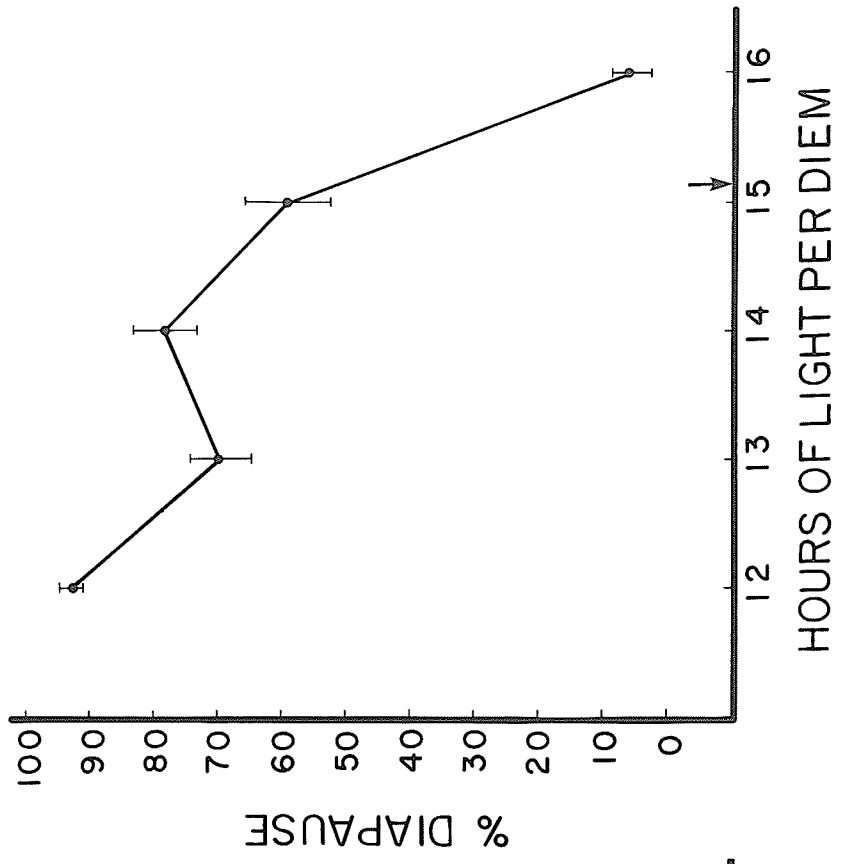
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Fig. 6

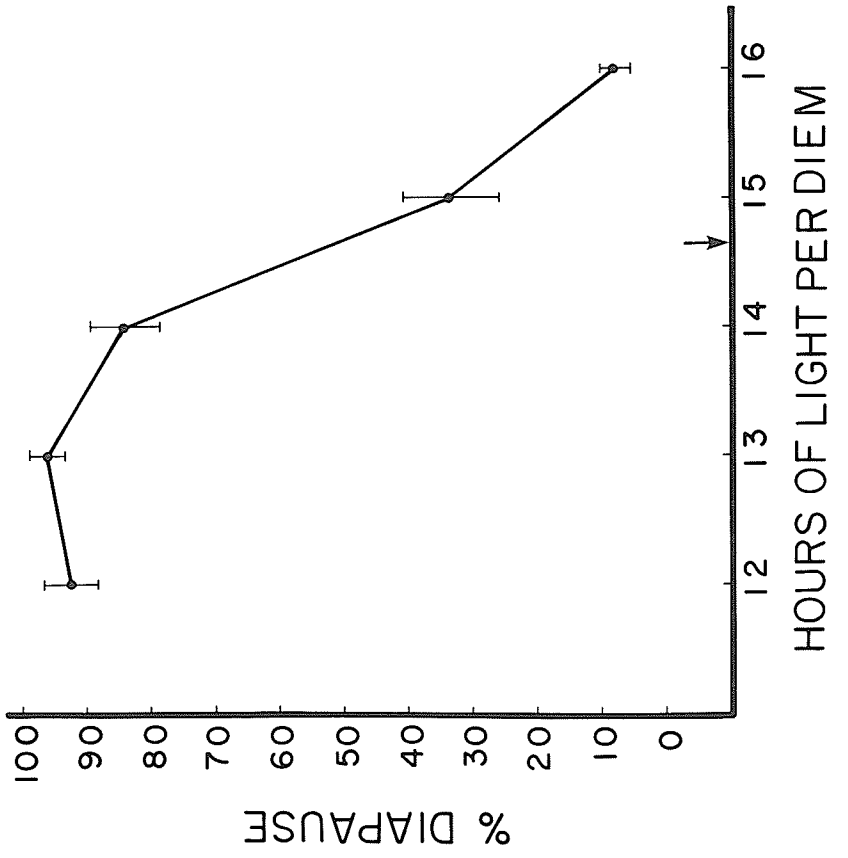
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Fig. 5

TABLE V

Effect of egg storage temperature on the incidence of diapause in the eggs of Ae.vexans females reared at different combinations of photoperiod and temperature. All eggs were maintained at 16L:8D for each storage temperature of 20°C and 23°C.

Rearing Photoperiod for females (L:D)	Rearing Temperature for females (°C)	Egg Storage Temperature (°C)	No. Egg Batches[1]	No. Viable Eggs	% Diapause Mean ± S.E.
12:12	23	23	29	1492	68.1 ± 4.27 *b*[2]
	23	20	10	539	92.5 ± 4.33*a*
	20	23	18	1100	52.3 ± 6.66 *c*
	20	20	28	1261	92.6 ± 1.70*a*
13:11	23	23	24	913	62.7 ± 6.17 *b*
	23	20	18	922	96.2 ± 2.85*a*
	20	23	21	1089	73.3 ± 5.58 *b*
	20	20	36	1389	69.4 ± 4.74 *b*
14:10	23	23	31	1340	47.1 ± 5.59 *b*
	23	20	24	1035	84.1 ± 5.54*a*
	20	23	33	1903	48.3 ± 5.71 *b*
	20	20	24	1013	78.2 ± 5.10*a*
15:9	23	23	24	1107	7.5 ± 3.23 *c*
	23	20	20	777	33.2 ± 7.69 *b*
	20	23	45	2452	7.4 ± 1.95 *c*
	20	20	30	1373	59.0 ± 6.71*a*
16:8	23	23	27	1060	2.1 ± 0.75 *b*
	23	20	19	812	7.5 ± 2.29*a*
	20	23	45	2439	1.0 ± 0.27 *b*
	20	20	29	1692	5.7 ± 3.25*ab*

1 Each egg batch represents the eggs oviposited by one female.

2 Common letter following column indicates no significant difference at 5% level between hatch response of eggs from Ae.vexans females kept at each photoperiod. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

at 20°C and the eggs stored at 20°C, the critical photoperiod was found to occur at ca. 15L:9D while for that population of which the P_1 females were held at 25°C and the eggs stored at 23°C, the critical photoperiod was determined to be ca. 13L:11D. The remainder of the populations examined exhibited an intermediate critical photoperiod value of ca. 14L:10D to 14½L:9½D. From the results of this experiment, it is difficult to determine whether the egg or the P_1 female stage of Ae. vexans is most influenced by ambient temperatures, with regard to the initiation of embryonic diapause at any given photoperiodic regime. It is interesting to note, however, that the greatest variability in egg hatch was found to occur at or near the point on the response curve determined to be the critical photoperiod.

(f) The incidence of diapause eggs from Ae. vexans females exposed to natural photoperiodic and temperature regimes.

From previous experiments, it was determined that the incidence of diapause in Ae. vexans was a factor attributable, primarily, to the influence of the temperature and photoperiodic regimes to which the P_1 female was subjected (Table IV), or the influence of the egg storage temperature (Table V).

This experiment was conducted in order to assess the phenology of embryonic diapause under natural photoperiodic and temperature regimes throughout the summer of 1979, and to determine, whenever possible, the influence of those parameters previously associated with embryonic diapause. The results of this experiment are summarized in Table VI. Relevant temperature and photoperiodic data are shown in Fig. 7.

A very low percentage (<5%) of the eggs oviposited by females

TABLE VI

The incidence of diapause in eggs from Ae.vexans females exposed to natural photoperiod and temperature regimes. All eggs were subjected to a hatching stimulus 21 days following oviposition.

Population	Group	Date of Blood meal	No. Egg Batches[1]	No. Viable Eggs	% Diapause Mean ± S.E.
I	A	8.VI.79	24	828	1.3 ± 0.75$_a$[2]
	B	15.VI.79	22	1106	1.7 ± 0.89$_a$
	C	22.VI.79	39	1579	3.0 ± 1.06$_a$
II	A	29.VI.79	32	1379	1.0 ± 0.47$_a$
	B	6.VII.79	35	1441	1.8 ± 0.66$_a$
	C	13.VII.79	42	1908	2.5 ± 1.04$_a$
III	A	13.VII.79	14	675	4.2 ± 1.96$_{ab}$
	B	20.VII.79	24	1160	3.5 ± 1.51$_a$
	C	27.VII.79	29	1073	1.6 ± 0.58$_a$
IV	A	27.VII.79	22	800	78.2 ± 5.29 *f*
	B	3.VIII.79	35	1412	82.8 ± 4.43 *f*
	C	10.VIII.79	32	1378	85.6 ± 4.63 *f*
IV	*B	3.VIII.79	19	799	45.3 ± 8.14 *d*
	*C	10.VIII.79	18	795	32.7 ± 6.23 *d*
V	**A	10.VIII.79	32	1950	19.5 ± 3.71 *c*
	**B	17.VIII.79	14	687	50.6 ± 11.63 *de*
VI	**A	24.VIII.79	19	1222	16.1 ± 5.49 *bc*
	**B	31.VIII.79	29	1519	40.3 ± 5.53 *d*
VII	***A	10.IX.79	34	1301	60.0 ± 3.74 *e*

* Eggs stored at 16L:8D and 23°C, rather than at natural photoperiodic and temperature regimes, for 21 days following oviposition.

** Adults held at 16L:8D and 23°C for 7 days prior to transfer to insectary.

*** Adults kept under a natural photoperiodic regime in the laboratory (23°C); eggs stored under similar conditions.

TABLE VI (continued)

1 Each egg batch represents the eggs oviposited by one female.

2 Common letter following column indicates no significant difference at 5% level between hatch response of eggs from females of different populations. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

Figure 7. Daylength curve (sunlight + civil twilight) and mean weekly temperatures recorded for Winnipeg, Manitoba (49^{O} 55'N - 97^{O} 05'W). Hours of daylight obtained from Smithsonian Meteorological Tables, 1966, Vol. 114; temperature recorded on Weksler ^{R} instrument placed within outdoor insectary.

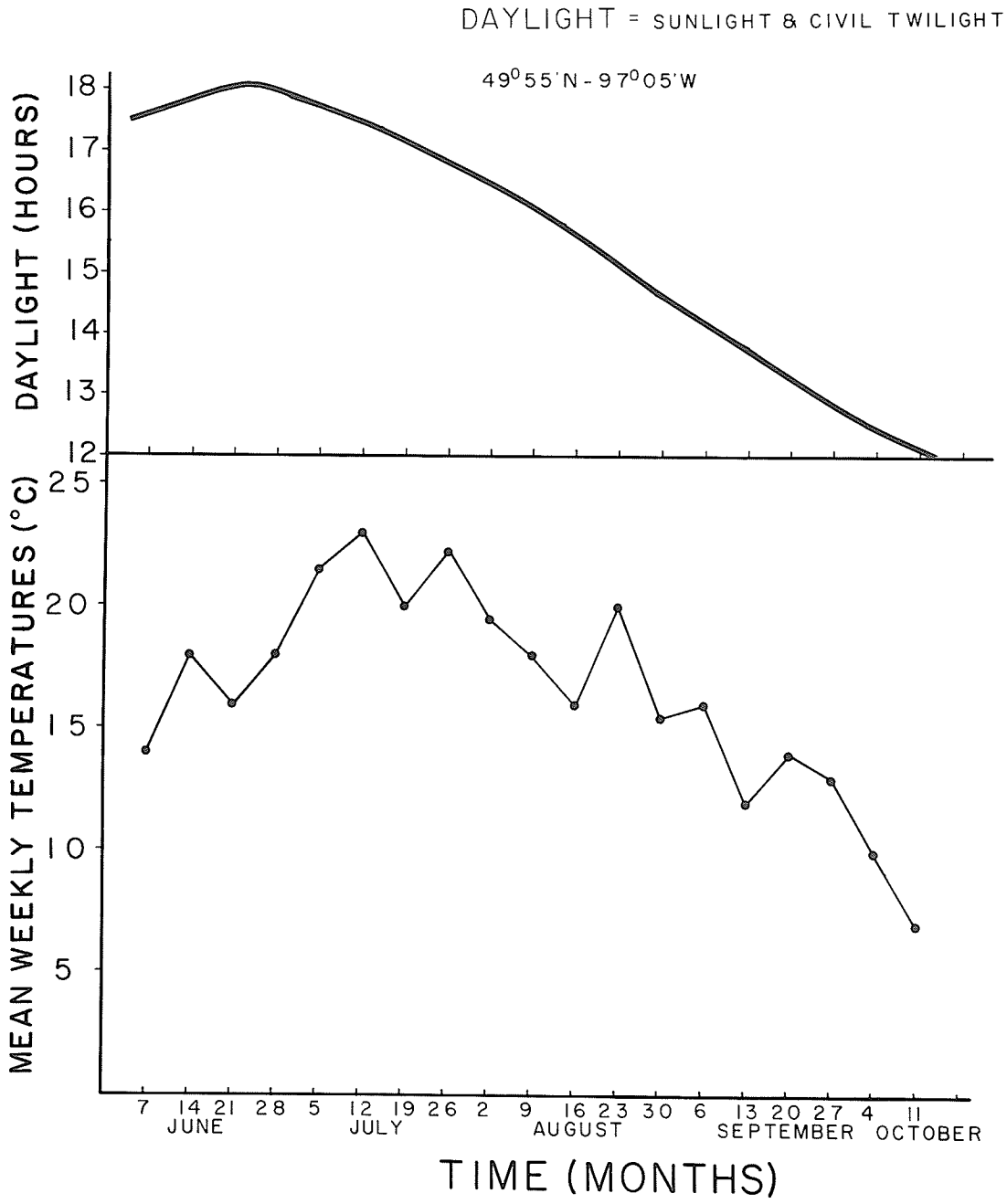
DAYLIGHT = SUNLIGHT & CIVIL TWILIGHT
49°55'N-97°05'W
DAYLIGHT (HOURS)
18
17
16
15
14
13
12
MEAN WEEKLY TEMPERATURES (°C)
25
20
15
10
5
7 14 21 28 5 12 19 26 2 9 16 23 30 6 13 20 27 4 11
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
TIME (MONTHS)

Fig. 7

throughout June and July (populations I, II, and III) were found to be in diapause, whereas the eggs produced by females of population IV, in early August, exhibited a very high percentage (>75%) diapause.

Eggs of population IV females (groups *B and *C), which had been returned to the laboratory for storage at 16L:9D and 23°C immediately following oviposition, exhibited a significantly higher percentage hatch than did those eggs of the same population which had been stored outdoors at a lower temperature. Nonetheless, the percentage egg diapause of population IV, groups *B and *C, was significantly higher than that found previously in either population I, II, or III.

Those females of populations V and VI, which had been maintained in the laboratory at 16L:8D and 23°C for seven days prior to transfer to the insectary, produced significantly fewer diapause eggs than did those females of population IV which had been maintained under a natural photoperiodic regime in the laboratory for seven days post-emergence. Group A females of both population V and VI produced significantly fewer diapause eggs than did group B females of either population.

Population VII females, maintained under natural photoperiodic conditions in the laboratory until oviposition was completed, produced significantly fewer diapause eggs than did females of population IV, groups A, B, and C, but significantly more diapause eggs than any other population with the exception of group B females of population V.

2. MAINTENANCE OF DIAPAUSE

The following experiments were performed to determine the effects of pre-treatment storage temperatures on diapause eggs, as well as the intensity of diapause in such eggs after storage for various periods of time.

(a) Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D) on diapause eggs of Ae. vexans.

As the results given in Table VII indicate, a very high percentage (>95%) of the eggs stored at 20°C remained in diapause even after 70 days, the duration of the experiment. There was no significant difference in the percentage hatch of eggs stored for periods of 28, 42, 56, or 70 days at 20°C, nor was a significant difference in egg hatch found between the two photoperiodic regimes used for this experiment.

At 23°C, a high percentage (>50%) of the eggs maintained at 16L:8D were found to hatch after 70 days, whereas those eggs maintained at 12L:12D were not found to hatch in significant numbers (>50%) after any storage period of less than 84 days. Although the 12L:12D photoperiod delayed egg hatch by about two weeks at 23°C (Table VII), under the preponderance of test conditions embryos were unresponsive to differences in photoperiod. After 84 days at 23°C, only a low percentage of the eggs remained in diapause at either photoperiod.

(b) Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D), as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae. vexans.

For this experiment, diapause eggs were obtained from field-collected Ae. vexans females captured during the late summer of 1978. The females were maintained under natural photoperiodic conditions in the laboratory and the deposited eggs were stored at 16L:8D and 20°C for twenty-eight days prior to induction into this experiment. A summary of the results for this experiment is presented in Table VIII and shown in Fig. 8.

While no significant difference in percentage egg hatch between 16L:8D and 12L:12D was observed after 7 and 14 days at 20°C, a

TABLE VII

Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D) on diapause eggs of Ae.vexans. All eggs held at 20°C or 23°C for designated period of time prior to immersion in hatching medium.

	Egg Storage Temperature (°C)							
	23				20			
	12L:12D		16L:8D		12L:12D		16L:8D	
Egg Storage (Days)	No. Viable Eggs	% Hatch[1] Mean ± S.E.	No. Viable Eggs	% Hatch[2] Mean ± S.E.	No. Viable Eggs	% Hatch[2] Mean ± S.E.	No. Viable Eggs	% Hatch[2] Mean ± S.E.
28	319	2.6 ± 1.90$_a$[3]	284	9.5 ± 3.74$_a$	250	1.8 ± 0.78$_a$	250	0.0 ± 0.00$_a$
42	303	21.7 ± 3.94 $_b$	269	23.5 ± 8.28$_a$	190	0.0 ± 0.00$_a$	183	0.0 ± 0.00$_a$
56	414	12.4 ± 3.10 $_b$	304	25.4 ± 8.03$_a$	201	2.5 ± 2.53$_a$	247	4.4 ± 4.44$_a$
70	566	19.9 ± 7.09 $_b$	359	[4]$_s$67.2 ± 7.92 $_{bc}$	242	0.0 ± 0.00$_a$	215	2.3 ± 2.34$_a$
84	380	63.8 ± 6.23 $_c$	413	53.8 ± 5.84 $_b$				
98	377	74.0 ± 4.70 $_c$	451	71.9 ± 6.75 $_{bc}$				
112	522	71.2 ± 6.38 $_c$	308	84.2 ± 7.67 $_c$				

TABLE VII (continued)

1 Mean and standard error of 10 individual egg batches examined.

2 Mean and standard error of 9 individual egg batches examined.

3 Common letter following column indicates no significant difference at 5% level between percentage egg hatch for different treatment periods. Student-Newman-Keuls test.

4 Letter *s* preceding column indicates a significant difference at 5% level between percentage egg hatch for different photoperiods at a given temperature. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

TABLE VIII

Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D), as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae. vexans. Eggs were maintained at 20°C for designated treatment periods prior to immersion in hatching medium. All unhatched eggs were subjected to the 14-day 'conditioning' period prior to a second hatch attempt.

	12L:12D			16L:8D		
No. Days at 20°C	No. Viable Eggs	% 1st Hatch[1] Mean ± S.E.	% Total Hatch[2] Mean ± S.E.	No. Viable Eggs	% 1st Hatch[1] Mean ± S.E.	% Total Hatch[2] Mean ± S.E.
7	135	[4] *d*10.2 ± 4.4*a*[3]	*d*10.2 ± 4.4*a*	137	*d*17.0 ± 3.0*a*	*d*18.0 ± 3.2*a*
14	125	*d*19.8 ± 3.1*ab*	*d*20.6 ± 3.2*a*	146	*d*31.5 ± 8.8*a*	*d*31.5 ± 8.8*a*
28	135	*d*17.3 ± 8.0*ab*	*e* 61.8 ± 7.4 *b*	129	*e* 68.3 ±11.1 *b*	*f* 92.2 ± 4.4 *b*
56	125	*d*32.7 ± 7.3 *b*	*e* 60.5 ±10.5 *b*	138	*f* 93.6 ± 3.8 *b*	*f* 95.2 ± 3.2 *b*
112	125	*d*59.8 ± 7.4 *c*	*e* 96.7 ± 2.6 *c*	144	*e* 89.6 ± 5.1 *b*	*e* 95.9 ± 2.9 *b*

1 Mean of 6 replicates; Egg hatch following treatment at 12L:12D or 16L:8D and 20°C for designated period.

2 Mean of 6 replicates; Total hatch = 1st Hatch + 2nd Hatch following a 14-day treatment at 16L:8D and 23°C.

3 Common letter following column indicates no significant difference at 5% level between percentage egg hatch for different treatment periods. Student-Newman-Keuls test.

TABLE VIII (continued)

4 Common letter preceding column indicates no significant difference at 5% level between percentage egg hatch at different photoperiods for each treatment period. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

Figure 8. Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D) at 20°C, as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae. vexans.

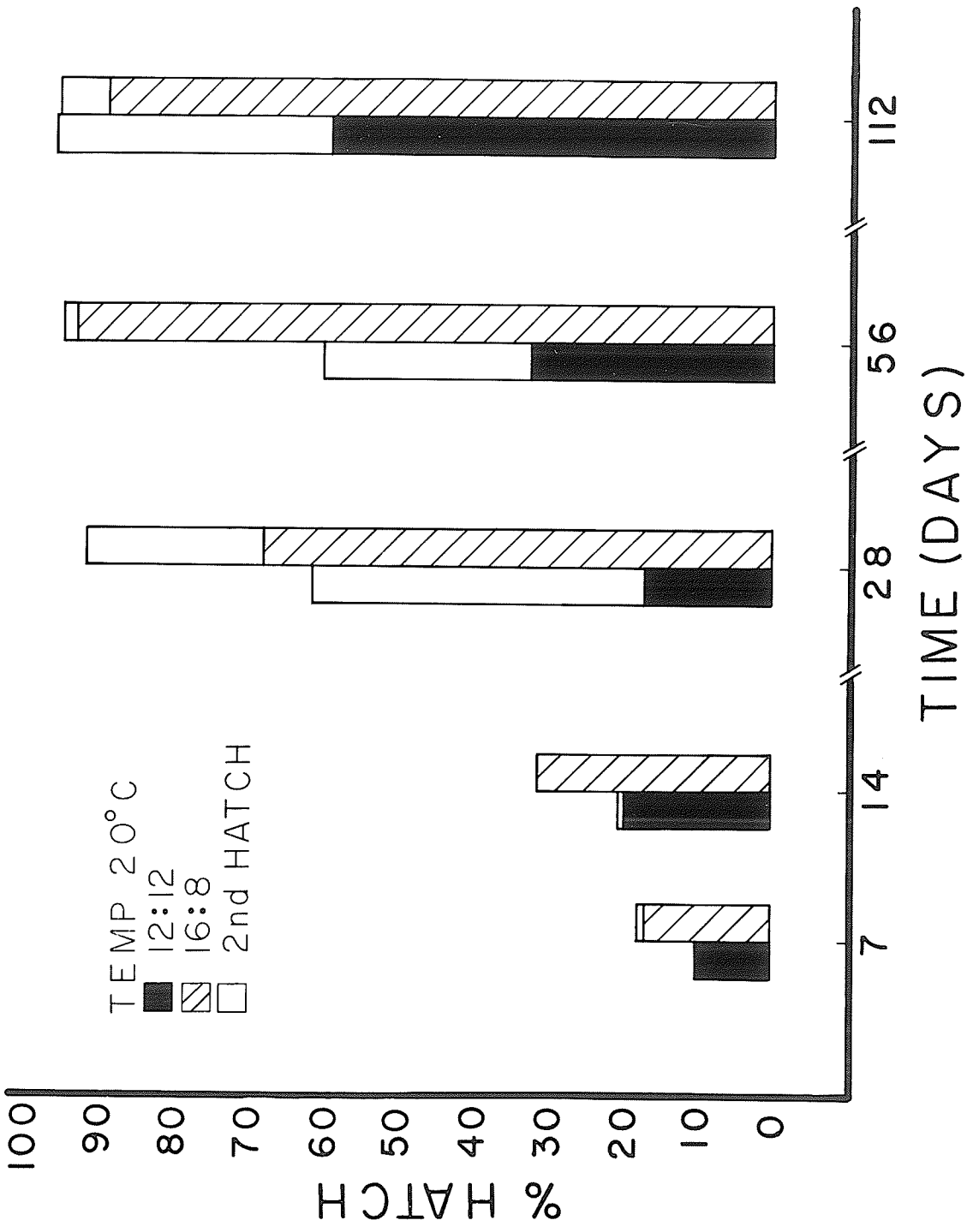
% HATCH
100
90
80
70
60
50
40
30
20
10
0
TIME (DAYS)
7
14
28
56
112
TEMP 20°C
12:12
16:8
2nd HATCH

Fig. 8

significantly greater number of eggs hatched after exposure to the long-photoperiod for 28, 56, and 112 days than did after exposure to a short-photoperiod for the same treatment periods. At each photoperiod, the percentage egg hatch increased with the length of the treatment period; a high percentage (>50%) of those eggs stored at 16L:8D and 20°C hatched after 28 days exposure, whereas at 12L:12D, a treatment period of 112 days was required before a significant proportion of the eggs could be induced to hatch.

The results of the 14-day post-treatment period revealed no significant difference between percentage hatch for those eggs stored at 16L:8D and 12L:12D for either 7 or 14 days. However, a significant increase in hatch was noted for eggs from both photoperiod treatments which had been subjected to a post-treatment of 16L:8D and 23°C after storage at 20°C for 28 days. Although no significant increase in hatch, due to the 14-day post-treatment, was found for eggs which had been stored at 16L:8D and 20°C for periods of 56 or 112 days, a significant increase in hatch did occur as a result of the post-treatment of eggs which had been stored at 12L:12D and 20°C for the same treatment periods. The response of the 12L:12D eggs, which had been stored at 20°C for 112 days, after the 14-day post-treatment period was not significantly different from the response of the 16L:8D eggs which had experienced the 14-day post-treatment following 28, 56 and 112 days storage at 20°C.

3. TERMINATION OF DIAPAUSE

(a) Effect of a low temperature (5°C), as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs Ae. vexans.

All diapause eggs were obtained from field-collected Ae. vexans females captured during the late summer of 1978. Following deposition,

the eggs were stored at 16L:8D and 20°C for 28 days prior to initiation of this experiment.

The primary objective of this experiment was to ascertain the effect of a low temperature (5°C) on the diapause condition in Ae. vexans eggs. Secondary objectives were: i) to determine the influence of a long-photoperiod (16L:8D) and a short-photoperiod (12L:12D) on embryonic diapause at a low temperature; and ii) to assess the effect of a 14-day post-treatment at 16L:8D and 23°C on any unhatched eggs following each low temperature treatment period. The results of this experiment are summarized in Table IX and shown in Fig. 9.

The length of storage time at 5°C had a significant effect on the percentage hatch of Ae. vexans eggs. A very high percentage (>85%) of the eggs, maintained at either photoperiod, remained in diapause for at least 56 days. However, after 112 days at 5°C, less than 5% of the eggs were found to remain in diapause. No significant difference in percentage hatch existed between eggs stored at either of the two photoperiods, except at the 28-day sample period where only a slightly significant difference was observed. The effect of photoperiod on diapause eggs appeared to be masked at the low temperature.

Although the 14-day post-treatment produced no significant increase in hatch following the 7- and 14-day low temperature treatment, a significant difference between the first and second hatch was very apparent following the 28- and 56-day treatment. This response to the 14-day post-treatment was not unlike that shown in Table VIII for eggs which had been treated at 12L:12D and 20°C for periods of 28 or 56 days prior to the post-treatment period. No effect on egg hatch, due to the 14-day

TABLE IX

Effect of a low temperature (5°C), as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae.vexans. Eggs were maintained at a photoperiod regime of either 12L:12D or 16L:8D for a designated treatment period at 5°C prior to immersion in a hatching medium. All unhatched eggs were subjected to the 14-day 'conditioning' period prior to a second hatch attempt.

	12L:12D			16L:8D		
No.Days at 5°C	No. Viable Eggs	1st % Hatch[1] Mean ± S.E.	% Total Hatch[2] Mean ± S.E.	No. Viable Eggs	1st % Hatch[1] Mean ± S.E.	% Total Hatch[2] Mean ± S.E.
7	122	[4] $_{d}$1.0 ± 1.0$_{a}$[3]	$_{d}$1.0 ± 1.0$_{a}$	128	$_{d}$0.0 ± 0.0$_{a}$	$_{d}$3.0 ± 2.0$_{a}$
14	129	$_{d}$4.0 ± 1.0$_{a}$	$_{d}$5.0 ± 1.2$_{a}$	119	$_{d}$5.0 ± 2.0$_{ab}$	$_{d}$5.0 ± 2.0$_{a}$
28	105	$_{e}$ 6.7 ± 0.7 $_{b}$	$_{f}$ 75.3 ± 6.3 $_{b}$	131	$_{d}$2.3 ± 2.3$_{ab}$	$_{f}$ 83.4 ± 2.0 $_{b}$
56	134	$_{d}$4.7 ± 2.4$_{ab}$	$_{e}$ 70.0 ±12.1 $_{b}$	121	$_{d}$10.3 ± 4.5$_{ab}$	$_{e}$ 74.7 ± 9.4 $_{b}$
112	136	$_{d}$97.8 ± 1.4 $_{c}$	$_{d}$98.5 ± 1.0 $_{c}$	129	$_{d}$99.4 ± 0.6 $_{c}$	$_{d}$100.0 ± 0.0 $_{c}$

1 Mean of 6 replicates; Egg hatch following treatment at 12L:12D or 16L:8D and 5°C for designated period.

2 Mean of 6 replicates; Total hatch =1st Hatch + 2nd Hatch following a 14-day treatment at 16L:8D and 23°C.

3 Common letter following column indicates no significant difference at 5% level between percentage egg hatch for different treatment periods. Student-Newman-Keuls test.

TABLE IX (continued)

4 Common letter preceding column indicates no significant difference at 5% level between percentage egg hatch at different photoperiods for each treatment period. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

Figure 9. Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D) at 5°C, as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae. vexans.

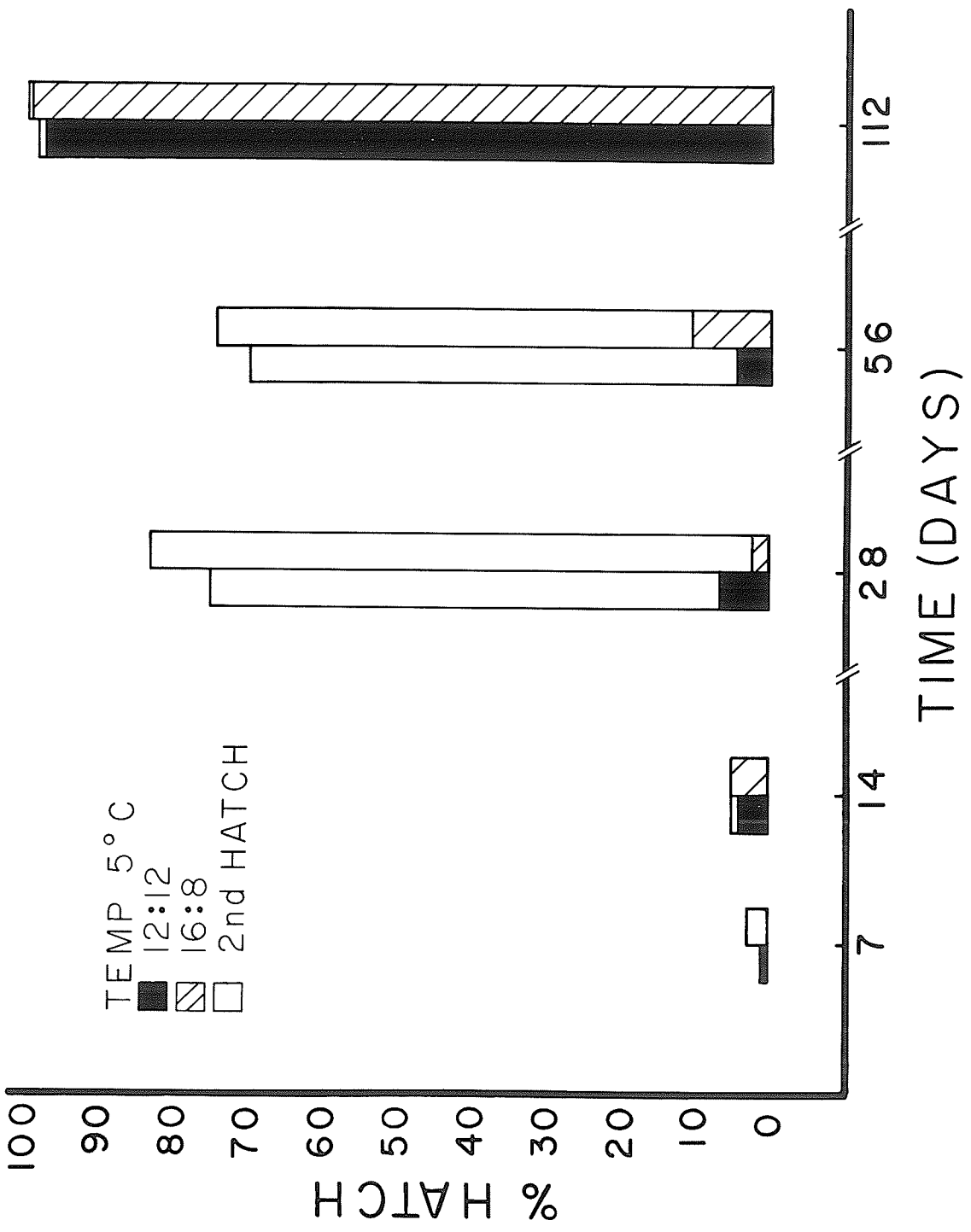
% HATCH
100
90
80
70
60
50
40
30
20
10
0
TIME (DAYS)
7
14
28
56
112
TEMP 5°C
12:12
16:8
2nd HATCH

Fig. 9

post-treatment, was noted following the 112-day treatment at 5°C since the percentage hatch was very high (>95%) for the initial flooding.

Egg viability was not adversely affected by storage of diapause eggs at 5°C for a period of up to 112 days.

(b) Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D), in combination with a high temperature (30°C), on diapause eggs of Ae. vexans.

Diapause eggs which had been obtained from 12L:12D females maintained at 23°C, and subsequently stored at 12L:12D and 23°C for 28 days, were used for this experiment. Prior to the experiment, all eggs were flooded in nutrient broth to eliminate any non-diapause eggs from the samples. In order to determine if a simultaneous influence of high temperature and long-photoperiod could increase the percentage hatch in diapausing eggs, batches of eggs were subjected to a temperature of 30°C and a photoperiodic regime of either 12L:12D or 16L:8D for treatment periods of 7, 14, 21, and 28 days.

The results of the experiment are given in Table X and indicate that, at a high temperature, there was no significant difference in percentage hatch between eggs maintained at 12L:12D and 16L:8D. Exposure of diapause eggs to 30°C for a treatment period of 7 days or more resulted in the termination of the diapause condition in a high percentage (>80%) of the eggs at either photoperiod. The termination of embryonic diapause appeared to be dependent upon exposure to a high temperature rather than exposure to a long-photoperiod (as previously shown in Table VII), i.e. the influence of photoperiod is subordinate to the effect of high temperature.

TABLE X

Effect of short-photoperiod (12L:12D) and long-photoperiod (16L:8D), in combination with a high temperature (30°C), on diapause eggs of Ae. vexans. Following a designated treatment period, all eggs were immersed in a hatching medium.

	12L:12D			16L:8D		
No. Days at 30°C	No. Egg Batches[1]	No. Viable Eggs	% Hatch Mean ± S.E.	No. Egg Batches	No. Viable Eggs	Mean ± S.E.
7	7	405	83.7 ± 4.90*a*[2]	7	359	83.2 ± 5.91*a*
14	6	323	96.9 ± 2.19 *b*	7	392	97.1 ± 1.27 *b*
21	6	272	98.9 ± 0.71 *b*	7	378	99.1 ± 0.46 *b*
28	6	416	98.9 ± 0.95 *b*	7	421	99.2 ± 0.56 *b*

1 Each egg batch represents the eggs oviposited by one female.

2 Common letter following column indicates no significant difference at 5% level between percentage egg hatch for different treatment periods. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

(c) Effect of a high temperature (30°C), as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae. vexans.

Diapause eggs, obtained from field-collected Ae. vexans females captured during the late summer of 1978, were used for this experiment. Following oviposition, the eggs were maintained at 16L:8D and 20°C for 28 days prior to induction into the experiment.

The purpose of this study was: i) to determine if a high temperature of 30°C could terminate diapause as readily in eggs which had been stored at 20°C as was found for eggs which had been stored at 23°C (as previously shown in Table X); ii) to determine the longevity of eggs stored at a high temperature of 30°C; and iii) to assess the effect of a 14-day post-treatment of 16L:8D and 23°C on any unhatched eggs following the initial hatch attempt.

As results given in Table XI and Fig. 10 indicate, there was no significant difference in egg hatch between 12L:12D and 16L:8D photoperiods at 30°C. A very high percentage (>85%) of the eggs hatched after 14 days at 30°C. An equally high percentage (>80%) of those eggs which had been stored at 23°C hatched after only 7 days at 30°C (Table X).

While there was no indication of embryonic mortality within the first 56 days of the experiment, it was found that only a few embryos were able to survive a storage period of 112 days at 30°C, at either photoperiod. In most instances, those embryos which were able to survive the storage period died during, or shortly after, hatching from the eggs.

TABLE XI

Effect of a high temperature (30°C), as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae.vexans. Eggs were maintained at a photoperiod regime of either 12L:12D or 16L:8D for a designated treatment period at 30°C prior to immersion in a hatching medium. All unhatched eggs were subjected to a 14-day 'conditioning' period prior to a second hatch attempt.

	12L:12D			16L:8D		
No. Days at 30°C	No. Viable Eggs	% 1st Hatch[1] Mean ± S.E.	% Total Hatch[2] Mean ± S.E.	No. Viable Eggs	% 1st Hatch[1] Mean ± S.E.	% Total Hatch[2] Mean ± S.E.
7	126	42.5 ± 8.3*a*[3]	62.9 ± 5.8*a*	136	42.4 ± 7.1*a*	56.3 ± 9.7*a*
14	111	95.0 ± 2.4 *b*	97.3 ± 1.8 *b*	130	87.2 ± 4.7 *b*	92.7 ± 2.7 *b*
28	128	98.9 ± 1.0 *b*	100.0 ± 0.0 *b*	121	96.7 ± 1.6 *b*	100.0 ± 0.0 *b*
56	121	99.2 ± 0.8 *b*	99.2 ± 0.8 *b*	126	96.9 ± 2.0 *b*	99.4 ± 0.6 *b*
112	dead embryos	-	-	dead embryos	-	-

1 Mean of 6 replicates; Egg hatch following treatment at 12L:12D or 16L:8D and 30°C for designated period.

2 Mean of 6 replicates; Total hatch = 1st Hatch + 2nd Hatch following a 14-day treatment at 16L:8D and 23°C.

3 Common letter following column indicates no significant difference at 5% level between percentage egg hatch for different treatment periods. Student-Newman-Keuls test.

Abbreviation: S.E.= standard error.

Figure 10. Effect of a short-photoperiod (12L:12D) and a long-photoperiod (16L:8D) at 30°C, as well as a 14-day post-treatment period at 16L:8D and 23°C, on diapause eggs of Ae. vexans.

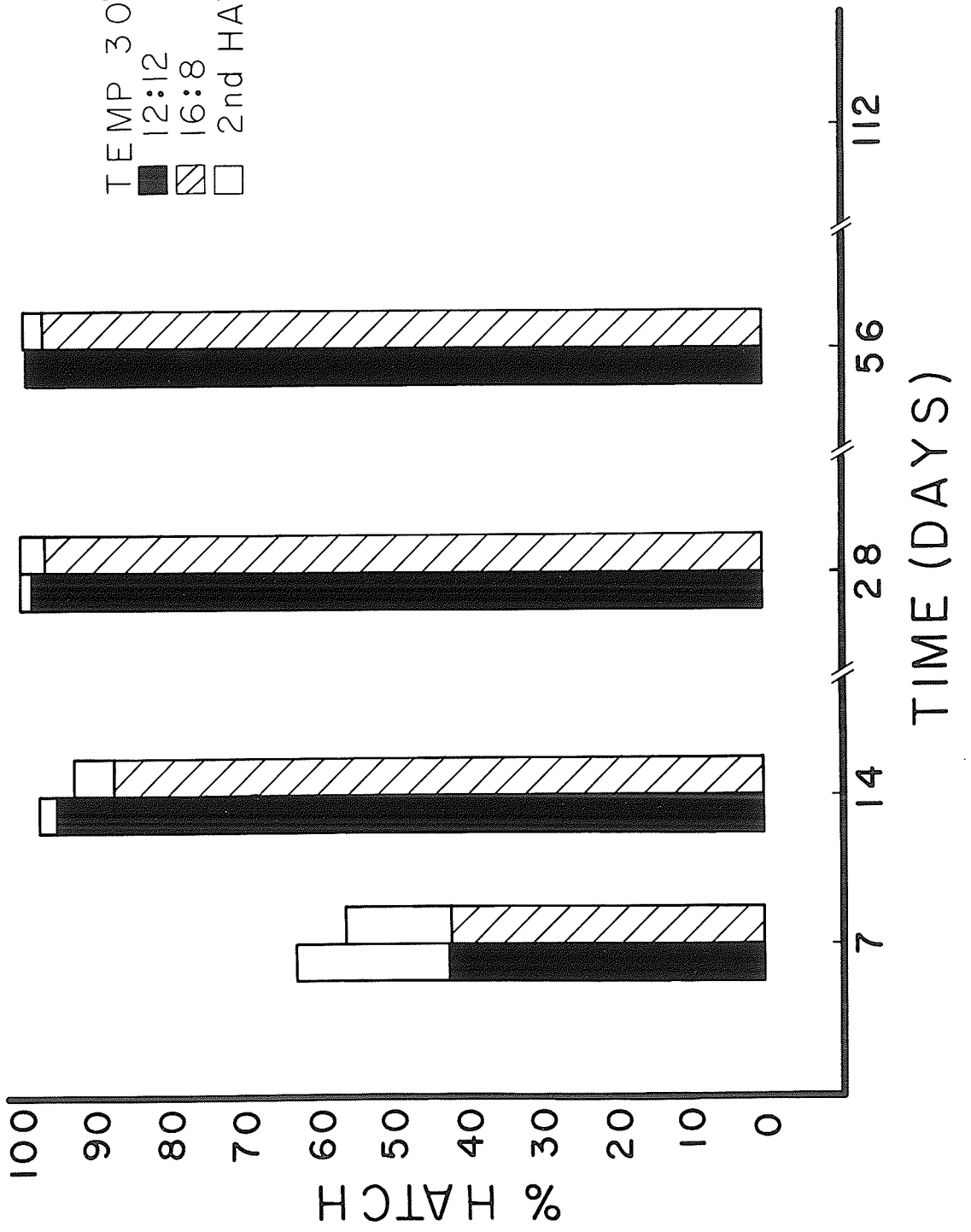
TEMP 30°C
12:12
16:8
2nd HATCH
% HATCH
0
10
20
30
40
50
60
70
80
90
100
7
14
28
56
112
TIME (DAYS)

Fig. 10

There was no significant increase in egg hatch as a result of a second hatch attempt. The 14-day post-treatment effect on unhatched eggs was subordinate to the high temperature treatment.

CHAPTER V

DISCUSSION

INDUCTION OF DIAPAUSE

Many investigators have demonstrated that the effect of photoperiod is cumulative and that often the photoperiodically sensitive stage of an insect precedes the stage associated with diapause. Various mosquito species have been found to exhibit a similar diapause induction response.

From the results of the experiments summarized in Table I, it is evident that diapause induction in Ae. vexans eggs is the result of a cumulative effect of photoperiodic influence on the preceding developmental stages. For the most part, these data compare favorably with that obtained by Wilson and Horsfall (1970) and show that the P_1 female is the stage most sensitive to day-length. However, there is evidence to suggest that the pupal stage, as well as the adult stage, exhibits a photoperiodic response. It is noted that, for the higher temperature treatment (23°C), a significantly higher incidence of diapause eggs was obtained from 16L:8D females which had been subjected to a short-photoperiod as pupae than was obtained from 16L:8D females which has been subjected to a long-photoperiod during the pupal stage. As stated by de Wilde (1962), photoperiodic induction of diapause is mostly reversible in the sensitive stage; photoperiods which induce diapause are more easily reversed by photoperiods which promote further development than in the inverse case. It is to be expected, if such is the case, that the incidence of diapause eggs will be partially determined by the photoperiod to which the Ae. vexans pupae are subjected. It is

perhaps appropriate that no evidence of pupal sensitivity is apparent in the lower temperature treatment (20°C), since the prolonged exposure (due to a slower developmental rate) of adults to a given photoperiod at the lower temperature would facilitate sufficient time (required day number, as discussed by Saunders 1976, and Beach 1978) for compliance with the influencing photoperiod.

The effect of temperature on the incidence of diapause in Ae. vexans eggs has been demonstrated by others (Wilson and Horsfall 1970; McHaffey 1972b) and is apparent from the data provided in this thesis. Despite only a slight difference in the range of temperatures (3°C) used in my experiments the higher temperature (23°C) caused diapause to be averted in a portion of the eggs examined, while the lower temperature (20°C) served to enhance the diapause response.

While it is evident, from my experiments, that diapause is averted in a significant portion of those eggs oviposited and maintained at 23°C, the results of the repeated-flooding experiment (Table II) show that those eggs which had exhibited a diapause condition following the first hatch attempt could not be induced to hatch during subsequent hatch attempts. It would appear that the diapause condition is relatively well-established and stable, in such eggs, after a 21-day post-oviposition storage period. Conversely, the data in Table II also illustrate the fact that eggs obtained from Ae. vexans females which are reared and conditioned under controlled conditions will hatch in toto when subjected to an appropriate hatching stimulus. Whereas Ae. vexans eggs which have been obtained from natural oviposition sites in the field usually exhibit a variable hatching response (Wilson and Horsfall 1970; Horsfall et al. 1973), little or no incidence of installment hatching was found in

laboratory material throughout the course of my experiments.

Further to the effect of temperature on the incidence of embryonic diapause, as discussed previously, the onset of embryonic diapause was found also to be directly related to the post-oviposition storage temperature as well as to the length of the post-oviposition storage period (Table III). Generally, the expression of embryonic diapause was found to occur within a shorter period of time and in a greater number of eggs at the lower temperature (20°C), as compared to the results of the higher temperature storage (23°C). The results of the 16-, 18-, and 21-day post-oviposition storage period not only demonstrate the significant difference in the order of egg hatchability between the two temperature treatments, but also the degree of variability in hatch response exhibited by the eggs. It is presumed, therefore, that a similar hatching response would be exhibited by eggs deposited in natural oviposition sites and could account for the erratic hatching tendencies of Ae. vexans eggs described by Horsfall et al. (1973).

The temperature-dependent response exhibited by eggs from mosquitoes reared under the influence of a short day-length has been shown to be directly related to the seasonal decrease of temperature in autumn. The photoperiodic and temperature adaptation by Ae. vexans adult females is further enhanced by a temperature adaptation by the deposited eggs. The ability of insect species to increase their frost-resistance, following the onset of diapause, as a result of the toughening action of low temperatures may be of ecological significance especially during winters of little snow or during severe temperature declines in autumn (Danilevskii 1965).

Ae. vexans exhibits what Beck (1968) refers to as a "Type I" induction curve (long-day response) in which relatively long daylengths tend to favor continuous growth while short daylengths favor diapause. Consistent with the concept of critical daylength provided by Beck (1968), the 50% response point on the population response curve was chosen to represent the critical daylength in my experiments. The incidence of diapause in eggs deposited by Ae. vexans females from different temperature and photoperiod treatments is plotted as diapause response curves shown in Figs. 1-5 (Data summarized from Tables IV and V). At all combinations of temperature treatments used, a high percentage of those eggs deposited by short-photoperiod females were induced to diapause. Conversely, a very low percentage of those eggs deposited by long-photoperiod females exhibited a diapause condition. The critical daylength was shown to be directly related to i) the temperature at which the females were reared and allowed to oviposit, and ii) the temperature at which the eggs were maintained prior to flooding. Ae. vexans females, maintained at a low temperature, are induced to oviposit diapause eggs at a longer daylength than are those females which are maintained at a higher temperature. The data from Table V coincide with and further augment those already obtained in support of the effect of egg storage temperature on the incidence of embryonic diapause. Photoperiodic induction of diapause in Ae. vexans populations varies within a broad range of photoperiods, with variation being accounted for, in large part, by ambient temperature conditions.

The findings of Breeland et al. (1965) may well illustrate the adaptations of geographically isolated Ae. vexans populations to photoperiodic induction of diapause. Even without the benefit of relevant

temperature data associated with the development of the light-sensitive stages of each population, it seems appropriate to regard the difference in the initial hatching response between the Minnesota and Alabama eggs to be, at least, partially attributable to variation in critical day-lengths characteristic for each population. The hatching resistance exhibited by Minnesota eggs during the early fall months lends support to the views of Danilevskii (1965) that the onset of diapause occurs at an earlier calendar date in northern zones due to an ecologically-unified mechanism whereby the photoperiodic induction of diapause increases as ambient temperatures decrease.

During at attempt to delineate the phenology of Ae. vexans populations within the immediate Winnipeg area, it was found that the onset of diapause occurred at an earlier calendar date than was originally anticipated for this species. The results of a preliminary investigation, carried out during the summer of 1978, revealed that a high incidence of diapause was apparent in eggs deposited in mid-August by wild-caught Ae. vexans females. A subsequent investigation, conducted during the summer of 1979 (Table VI), confirmed the onset of diapause in Ae. vexans eggs which were deposited in an outdoor insectary during the first week of August. It was determined that the expression of embryonic diapause was photoperiodically induced during the P_1 adult stage, while the incidence of diapause eggs was governed by egg storage temperatures. This conclusion is supported by data produced during laboratory experiments (Figs. 1-5).

A comparison of egg diapause between group C, pop. III and group A, pop. IV is of particular interest. Since the eggs of both groups were maintained under identical temperature and photoperiodic regimes prior

to flooding, the differences in egg diapause cannot be attributed to environmental influences on the egg stage. It is noted that, following a blood meal on July 27, 1979, the P_1 females of both groups were subjected to identical temperature and photoperiodic regimes, as were the eggs produced by the P_1 females of each group. Seemingly, the females of population III were influenced only by the daylength initially experienced (>critical daylength) and not by the shortened daylength (<critical daylength) experienced by the population IV females. If such is the case, it could be argued that the cumulative effect of a long-daylength, once well-established in an individual, is not altered or reversed by a continuously decreasing daylength. It is estimated that the maximum difference in daylength (sunlight plus civil twilight) experienced by females of the two populations was ca. 30 minutes. In consideration of the obvious differences in the incidence of diapause exhibited by the eggs from population III and population IV, there is reason to assume that the critical daylength is quite sharply defined and characteristically occurs prior to the last week in July for local Ae. vexans populations.

There is also evidence to suggest that moderately low (15-20°C) ambient temperatures were partially responsible for the high incidence of diapause in eggs of population IV. Eggs of population IV (groups *B and *C) which were maintained at 23°C, following oviposition, exhibited a significantly lower diapause incidence than did those eggs from population IV which were stored in the outdoor insectary.

During this investigation, an apparent reversal of photoperiodic influence was found to occur in a few groups of females which were

transferred to the outdoor insectary. While group A females of population V produced relatively few diapause eggs, such a response was not unexpected since these females had been maintained at a long-daylength (16L:8D) prior to transfer to the insectary. However, a significantly greater number of diapause eggs were produced by group B females which had been subjected to a 14-day exposure to natural temperature and photoperiodic regimes following an initial 7-day exposure to a 16L:8D photoperiod. It was concluded that a reversal of photoperiodic influence, in addition to a shift in critical daylength as a result of a lower ambient temperature, facilitated the induction response exhibited by group B females. Although the effect of fluctuating ambient temperatures on the female response cannot be confirmed, it is noteworthy (Fig. 7) that temperatures experienced in August were approximately equal to temperatures experienced in June; the incidence of diapause in June appeared not to be related to such ambient temperatures. Further, the response of population VI females were parallel to that of population V females despite the fact that females of population VI were subjected to temperatures which were noticeably lower.

To date, very little information has been provided regarding the light intensity thresholds to which the various developmental stages of mosquitoes are sensitive. A related study, conducted by Beach and Craig (1979), has indicated that Ae. atropalpus experiences an asymmetrical day since the subjective day began 1-2 hours before sunrise and ended less than 1 hour before sunset. The results of their study have implied that the diapause photoreceptor(s) perceived lower light levels at dawn than at dusk. Similar evidence of an asymmetrical day was provided by Bradshaw (1972) for Chaoborus americanus. The reduction in light

sensitivity at dusk was attributed to a partial bleaching of the pigments in the photoreceptor(s) during prolonged exposure to light (Bradshaw 1972).

Light intensity thresholds for photoperiodic reactions are generally regarded to be quite low (0.025-25 lux) for most insect species investigated; light intensities above this range appear to saturate the photoreceptor(s) thereby causing insects to respond in an identical fashion to either low light intensities or full sunlight (Saunders 1976). Since the thresholds are usually below that of the available light at dawn or dusk, the effective "natural photoperiod" which may be perceived by insects should include both calendar daylength and the two periods of civil twilight (de Wilde 1962; Saunders 1976). However, early illumination (dawn) may be rendered ineffective if morning temperatures are too low (de Wilde 1962).

In view of the perplexities associated with a defined light intensity threshold, it is difficult to compare measurements of critical daylength derived from laboratory studies on Ae. vexans with those derived under natural temperature and photoperiodic regimes. The accuracy of the estimated critical daylength found to occur in Ae. vexans populations maintained outdoors (Table VI) is supported by previous preliminary investigations on wild-caught Ae. vexans populations. There is little doubt that eggs, produced by Ae. vexans populations in the Winnipeg area during the latter portion of the month of July, will exhibit a low order of hatchability due to the onset of diapause. Certainly, consideration must be given to ambient temperatures in order to assess the period of time required for expression of the diapause condition in the egg stage. A graph depicting daylength (inclusive of

civil twilights), shown in Fig. 6, provides a relationship between the natural photoperiod experienced at this latitude (49°55'N) and the data summarized in Table VI. To suggest that Ae. vexans is induced to diapause at any daylength less than 17 hours (July 20th) would not be consistent with the findings of previously described laboratory experiments nor with the findings of others (Smith and Brust 1971; Evans and Brust 1972; Taylor 1973; Kalpage and Brust 1974) for several mosquito species common to this latitude. There is reason to speculate, therefore, that Ae. vexans may also experience an asymmetrical day, as is suggested for Ae. atropalpus (Beach and Craig 1979), or be sensitive to a light intensity threshold which exceeds the range of that proposed by Saunders (1976). Experiments designed to elucidate the light intensity threshold of Ae. vexans populations are needed.

The fact that many multivoltine insect species are photoperiodically induced to enter a diapause condition during the summer months when temperature and other required conditions are favorable has been well documented. Clearly, it is evident that Ae. vexans must be numbered among those species exhibiting such a response. Although eggs deposited by long-photoperiod adults may demonstrate a reduced hatch after a 4-week exposure to temperatures in the 18°C range (Wilson and Horsfall 1970), the ability of these eggs to survive extended periods of adverse conditions may be described as variable. Unless such eggs are subjected to low temperatures soon after oviposition, considerable reserves of nutrition could be expended prior to the onset of diapause. The depletion of nutritional reserves could adversely affect the production and maintenance of certain biochemical products required during the diapause condition (Mansingh 1971). The ecological significance of early diapause,

characteristic of local Ae. vexans populations, is that the eggs undergo the appropriate physiological adjustments, including a reduced rate of metabolism, very soon after oviposition. Cold-hardiness, as well as the intensity and duration of diapause, is inversely proportional to the rate of metabolism, i.e. the lower the rate of metabolism, the greater is cold-hardiness, and intensity and duration of diapause (Mansingh 1971). Those eggs failing to enter diapause due to photoperiodic influence may not be prepared to withstand the cold autumn temperatures, before there is sufficient snowfall to insulate the eggs from the nightly low temperatures, nor the prolonged winter conditions lasting between 6-7 months.

MAINTENANCE OF DIAPAUSE

It has been pointed out by Horsfall et al. (1973) that latency could be induced in Ae. vexans eggs at all temperatures below 25°C. Further, a conditioning period was required to initiate a hatching response in eggs which had been held at a temperature in the 18-23°C range. During my photoperiodic induction experiments, it was found that eggs of local Ae. vexans populations could be induced to enter a diapause condition at moderate temperatures of 20 and 23°C. While the obvious differences in the incidence of egg diapause were attributed to the temperatures at which the eggs were maintained, no attempt was made previously to determine the duration of the diapause condition at either of the two temperatures.

The results of the initial experiment (Table VII) show that the duration of the diapause condition is affected by the temperature at which the eggs are maintained. While the 20°C treatment was terminated

after 70 days due to lack of sufficient egg numbers, there is evidence to suggest that the intensity of diapause was lower in those eggs stored at 23°C. In addition, there was no apparent photoperiodic effect noted on the diapause condition in the 20°C treatment, while in the 23°C treatment, the duration of diapause lasted ca. 2 weeks longer at the short-photoperiod condition than at the long-photoperiod condition. A similar photoperiodic effect on the duration of diapause was evident in the results of a 20°C treatment shown in Table VIII and Fig. 8. While eggs, subjected to the short-photoperiod, exhibited a gradual increase in hatch response which was directly related to the length of the storage period, a relatively spontaneous hatch response was demonstrated by the long-photoperiod eggs after a 28-day treatment period. Although the duration of egg diapause may be shortened by exposure to a long-photoperiod, this fact is of little consequence to local Ae. vexans populations which would experience not only a decrease in daylength below that of the critical daylength, but also a decline in ambient temperature (as shown in Fig. 6), after the onset of diapause.

The apparent differences in hatch response in eggs subjected to the 20°C treatments in Tables VII and VIII, can only be attributed to the pre-treatment conditions to which the eggs were subjected. Presumably, a decrease in storage temperature (from 23°C to 20°C) invoked a greater diapause intensity in those eggs used in the experiment reported in Table VII, whereas those eggs used in the experiment reported in Table VIII showed a hatch response consistent with a uniform storage temperature of 20°C.

In accordance with a 14-day conditioning period proposed by Horsfall et al. (1973) for the entrainment of a hatch response in

diapause eggs, it was found that after 56 days at 20°C (28 days pre-treatment + 28 days treatment) a significant increase in hatch response was demonstrated by eggs which were subjected to an additional 14-day storage period at 16L:8D and 23°C (Table VIII). The effects of such a post-treatment storage period were most pronounced for eggs previously held at the 12L:12D photoperiodic regime. The results of the experiment shown in Table VIII also indicate that the majority of eggs, maintained under a short-photoperiod at 20°C, remained in diapause for a period of at least 84 days (28 days pre-treatment + 56 days treatment). However, such eggs were predisposed to hatch after a period of 56 days (28 days pre-treatment + 28 days treatment) if subjected to a suitable conditioning treatment. It is suggested that diapause development, as described by Andrewartha (1952), is completed in Ae. vexans eggs, maintained at 20°C, within a period of 56 days following oviposition.

TERMINATION OF DIAPAUSE

Many investigators have demonstrated that prolonged exposure to low temperatures terminated the diapause condition in a number of insect species. In this laboratory, it has been observed that the embryonic diapause of a number of mosquito species, including Ae. vexans, could be terminated by subjecting the eggs to a temperature of 5°C for 3-4 months. This observation is in agreement with Horsfall (1956), Beckel (1958), Khelevin (1959), Horsfall and Fowler (1961), Brust and Costello (1969), Anderson (1970), McHaffey and Harwood (1970), and Ellis and Brust (1973), who have demonstrated that diapause is terminated even under dark conditions at low temperatures. The results shown in Table IX and Fig. 9 indicate that the influence of photoperiod on the termination of diapause

is masked by low temperatures as eggs hatched as readily at 12L:12D as at 16L:8D.

It is evident, in reviewing the results of the low temperature experiment, that a period of 56-112 days at 5°C was required to complete both the refractory and activated phase of the diapause condition in the eggs examined. While it is apparent that a 14-day conditioning period (16L:8D and 23°C) served to accelerate the activated phase of the diapause phase in eggs which had been held at 5°C for a minimum of 28 days, it is difficult to determine whether or not the refractory phase was influenced by the conditioning period. However, since there was no significant increase in hatch response after the 56-day treatment that would suggest a difference in the physiological state of the 28- and 56-day old eggs, it would seem reasonable to assume that the refractory phase was completed in the eggs within 28 days after exposure to a 5°C temperature. Thereafter, the eggs remained in an activated phase which, according to Mansingh (1971), could last for several months under field conditions but which could be terminated in the interim by exposure to favorable environmental conditions.

The results of the high temperature experiments, summarized in Tables X and XI, indicate that an elevation in temperature to 30°C accelerates the processes of diapause development (refractory phase) and neuroendocrine activity (activated phase) necessary for termination of diapause. High temperatures abbreviate the activated condition in diapausing individuals by increasing the relative rates of all the physiological and biochemical developmental processes (Mansingh 1971). Horsfall et al. (1973) have recognized that a conditioning period is essential for the entrainment of a hatching response in Ae. vexans

eggs known to be in a diapause state; while the diapause condition is eventually eliminated over time, a high temperature (≥25°C) will minimize the period of conditioning required.

When comparing the effects of high temperature on diapause eggs of different temperature backgrounds (Tables X and XI), there is some evidence to suggest that diapause is terminated more slowly in eggs which were previously held at the lower temperature (20°C). The difference in hatch response between the two groups of eggs, which was noted only for the 7-day treatment period at 30°C, may be attributed to the difference in diapause intensity previously noted to occur in eggs at the two temperatures (Tables I and III). Seemingly, such a difference in hatch response may appear irrelevant in light of the fact that eggs from both groups demonstrated a very high order of hatch after exposure to 30°C for only 14 days. However, it is anticipated that eggs obtained from natural oviposition sites during autumn will be found to exhibit a wider range of diapause intensity than was found in eggs used in these experiments. If such is the case, field-collected eggs will probably require an extended period of exposure (>7 days) to a high temperature (30°C) in order to effect an *in toto* hatch response. This occurrence may be of significant value to those investigators attempting to determine the distribution and relative abundance of *Ae. vexans* populations within a designated region by means of an egg sampling regime during the fall of the year.

While embryonic diapause can be terminated by exposure of the eggs to a high temperature for a short duration, a high incidence of embryo mortality occurs if eggs are subjected to the high temperature for an extended period of time (112 days). Since there was no evidence of

embryonic aestivation noted during these experiments, it is speculated that many embryos died as a consequence of nutritional reserves being depleted within the egg.

Unlike the results found at lower temperatures (20° and 23°C) in previous experiments, no significant increase in egg hatch occurred as a result of a second hatch attempt. The 14-day treatment (16L:8D and 23°C) of any unhatched eggs was subordinate to the initial hatch attempt following the high temperature treatment. An *in toto* hatch response was noted only after the eggs were subjected to the high temperature for an appropriate length of time.

Photoperiodic conditions did not affect the percentage hatch at a treatment temperature of 30°C. Although the influence of photoperiod on diapause termination appears to be masked at a high temperature, this is of little ecological significance as high temperatures could only occur during a season when daylength is favorable for growth conditions. Since cold temperatures have been shown to terminate diapause even before the appropriate photoperiod and temperatures occur in the spring, it is suggested that neither long-photoperiod nor high temperatures play a vital role in terminating diapause in the eggs of *Ae. vexans* under field conditions.

CHAPTER VI

SUMMARY

This study represents an effort to delineate the effects of temperature, photoperiod and maternal influence on the incidence of diapause in embryonated eggs of Ae. vexans. It is suggested that the findings of this study will provide a significant contribution to the existing literature related to the biology of Ae. vexans and, in addition, will provoke further insight and clarification regarding the role of diapause in the preservation and general prosperity of this, and perhaps other, mosquito species.

The following summary is intended to represent the order of findings, as discussed in the text of this thesis, and is not intended to represent any order of significance placed upon the findings:

1. Diapause induction in Ae. vexans eggs was found to be a cumulative effect of photoperiodic influence on the preceding developmental stages. P_1 females subjected to a short-photoperiod (12L:12D), during the interval between emergence and oviposition, produced a high percentage of diapause eggs while P_1 females subjected to a long-photoperiod (16L:8D) for a similar period of time produced very few diapause eggs at temperatures of 25°, 23°, and 20°C.
2. The induction of embryonic diapause in Ae. vexans is influenced not only by the temperature at which the eggs are maintained following oviposition but also by the temperature to which the P_1 females are exposed prior to oviposition. Embryonic diapause was most apparent, in the greatest number of eggs, when both the P_1 females and the deposited eggs were subjected to the lowest temperature tested (20°C). If either, or both, of these developmental

stages was subjected to a higher temperature (23°C), the incidence of embryonic diapause was lessened. Further, the onset of embryonic diapause occurred within a shorter period of time in eggs stored at the lower temperature.

3. The critical daylength, associated with the induction of diapause in 50% of the eggs produced by a population of Ae. vexans females, is directly related to the temperature at which the P_1 females are reared and allowed to oviposit as well as the temperature at which the eggs are maintained following oviposition. Ae. vexans females maintained at a low temperature, produce diapause eggs at a longer daylength than do those females which are maintained at a high temperature. Local populations of Ae. vexans were found to produce diapause eggs by late July.
4. The duration of the diapause condition in Ae. vexans eggs is determined by the temperature at which the eggs are maintained. Diapause intensity is greater in those eggs stored at lower temperatures. Further, the duration of egg diapause is shortened by exposure to a long-photoperiod, particularly at a higher temperature (23°C versus 20°C). A significant proportion of diapause eggs, maintained at 12L:12D and 20°C, are predisposed to hatch after a period of 56 days if subjected to a suitable conditioning treatment of 16L:8D and 23°C for 14 days. This evidence suggests that the "diapause development" processes (Andrewartha 1952) in Ae. vexans eggs are completed within 56 days at 20°C.
5. The influence of photoperiod on the termination of embryonic diapause is masked at both low and high temperatures of 5°C and 30°C, respectively. That is, the effect of photoperiod is

subordinate to the effect of either low of high temperatures. Embryonic diapause is terminated within a period of 112 days at 5°C, however diapause can be culminated in a significantly high percentage of eggs after only 28 days at 5°C provided that the eggs are conditioned at 16L:8D and 23°C for 14 days after the low temperature treatment. Diapause eggs, held at 30°C for a period of 7-14 days, hatch *in toto* when immersed in nutrient broth solution.

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